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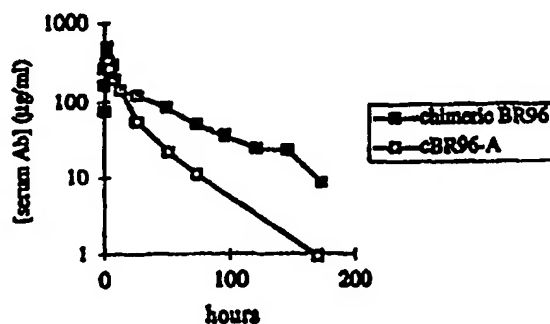
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(54) Title: A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS



Plasma clearance in high LeY expressing dogs chimeric versus constant region mutant of cBR96-2.

(57) Abstract

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an immunoglobulin or Ig fusion protein molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by inactivation of at least a portion of the constant region.

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5    **A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED  
TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN  
THERAPY AND IN VIVO DIAGNOSIS**

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10   Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

15   **TECHNICAL FIELD OF THE INVENTION**

         The present invention relates to methods for inhibiting or reducing immunoglobulin-induced toxicity resulting from therapy or in vivo diagnosis. Specifically, in lieu of using unmodified antibodies or recombinant binding proteins for in vivo use, the  
20   invention provides the use of modified antibodies or recombinant binding proteins which have been structurally altered in the constant domain so that upon administration immunoglobulin-induced toxicity is reduced or inhibited.

**BACKGROUND OF THE INVENTION**

25

         Over the years investigators have attempted to harness the immune system for therapeutic use. Immunoglobulin (Ig) molecules which constitute an important part of the immune system are of great interest because they (1) react with a diverse family of ligands, (2) possess different effector functions and (3) are of great  
30   biological importance. Despite its potential, a persistent problem with

immunoglobulin immunotherapy has been, among other problems, the toxic effect to normal cells of using antibodies which recognize both normal and diseased cells. This problem is far-reaching because the majority of antibodies presently available recognize a target located on both normal and diseased cells (Slavin-Chiorini, et al.,  
5 Int. J. Cancer 53: 97-103 (1993)).

The constant region can promote cell death through antibody dependent cell mediated cytotoxicity (ADCC) or by complement dependent cytotoxicity (CDC). Despite the deletion of portions of the constant region, particularly the CH<sub>2</sub> domain,  
10 the antigen binding function can be retained (D. Yelton, M. Scharf, Mutant monoclonal antibody with alterations in biological functions, J. Exp. Methods 156:1131-1148 (1982)).

Others have generated a CH<sub>2</sub>-deleted antibody (Mueller et al., Proc. Natl. Acad. Sci. USA 87: 5702-5705 (1990)). Their findings provide that the CH<sub>2</sub>-deleted antibody  
15 was cleared from the blood of tumor-bearing mice much faster than the corresponding intact antibody. Other in vivo findings also confirmed that a CH<sub>2</sub>-deleted antibody, designated ch14.18DCH2, is a potentially useful reagent for radioimmunodetection of human tumors because of its reduced immunogenicity,  
20 increased target specificity, and rapid clearance from circulation (Mueller et al., Proc. Natl. Acad. Sci. USA 87: 5702-5705 (1990)).

Generally, whole antibody molecules are composed of two heavy (H) and two light (L) chains which are held together by covalent bonds (disulfide) and non-covalent  
25 interactions. Each chain contains a variable region (V) and a constant region (C). The variable regions at the amino termini of the two chains form the antigen binding region. The constant region of the H chain has three components or domains. Occasionally, the first constant region domain (CH<sub>1</sub>) interacts with the C region of the L chain through hydrophobic interactions and generally a disulfide bond,



depending on isotype. The next C region stretch is the hinge-acting disulfide bond stably introduced between two H chains. The second constant region domain (CH<sub>2</sub>) is adjacent to the hinge region. CH<sub>2</sub> contains sequences important for effector functions of the antibody, such as the sequences responsible for complement  
5 fixation, and Fc receptor binding. The third constant region domain (CH<sub>3</sub>) is located at the carboxyl terminus of the H chain, and is considered to play an important role in H chain assembly as well as some C region functions.

Today many antibodies in clinical trials are directed against tumor associated  
10 antigens. Most tumor associated antigens are not tumor specific but are also generally found on the cell surface of some normal, non-tumorigenic cells. The clinical use of some antibodies directed against tumor associated antigens are limited because of the toxicity associated with their use. Therefore, there is a need for methods for inhibiting toxicity associated with immunoglobulin use in the field of  
15 disease therapy (e.g., therapy for tumors, kidney disease, and the like) and in vivo diagnosis.

We addressed this need by discovering methods for inhibiting or reducing toxicity to normal cells generally associated with immunoglobulin immunotherapy or in vivo  
20 diagnosis, wherein the immunoglobulin recognizes both diseased and normal cells. Our discovery involves generating immunoglobulin molecules or Ig fusion proteins having structurally altered constant regions which inhibit or reduce immunoglobulin-induced toxicity.

## 25 SUMMARY OF THE INVENTION

The present invention provides methods for inhibiting immunoglobulin-induced toxicity by using known immunoglobulin or Ig fusion protein molecules which are structurally altered in their constant regions so that the resulting structurally altered

immunoglobulin or Ig fusion protein molecules exhibit reduced or inhibited toxicity in vivo compared to their original unmodified counterparts.

5 Structural alteration of the constant region may be effected in a number of ways as long as it results in reducing or inhibiting immunoglobulin-induced toxicity.

In accordance with the practice of the invention, structural alteration of the constant region is effected by deletion of the entire constant region. In another embodiment, only the CH<sub>2</sub> domain is deleted. In another embodiment, only that portion of the  
10 CH<sub>2</sub> domain that binds the Fc receptor is deleted. In yet another embodiment, only that portion of the CH<sub>2</sub> domain that binds the complement component C1q is deleted. Alternatively, in another embodiment, multiple deletions in discrete Fc receptor and complement component binding domains are effected.

15 Alternatively, structural alteration is effected by single or multiple mutations in the CH<sub>2</sub> domain such as amino acid insertions and substitutions. The mutation or mutations must result in inhibiting immunoglobulin-induced toxicity. By way of example, the amino acids in multiple toxicity associated domains in the constant region can be altered so as to render the constant region unable to mediate a ADCC  
20 response or activate complement thereby inhibiting immunoglobulin induced toxicity resulting from immunotherapy. Alternatively, multiple amino acids in a single toxicity associated domain in the constant region can be altered.

Further alternatively, structural alteration can be effected by isotype switching  
25 resulting in an altered immunoglobulin molecule that either does not induce toxicity or induces some limited toxicity but does not cause a harmful effect. For example, isotype switching can result in the constant region being unable to mediate a CDC or ADCC response or some other activity which mediates toxicity.

**BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 is a line graph showing plasma clearance in high Le<sup>y</sup> expressing dogs using chimeric BR96 versus constant region mutant of cBR96-2.

5

Figure 2 is a schematic diagram of a plasmid designated pTWD-cJVK.L1 including the chimeric (c)BR96-light chain (SEQ ID NO. 11).

Figure 3 is a schematic diagram of a plasmid designated pD16hJ1.L1 including the  
10 human (h)BR96-light chain (SEQ ID NO. 13).

Figure 4 is a schematic diagram of a plasmid, designated pD17-hJm14-dCH2.H1, of hBR96-2A (i.e., human mutant BR96 having the H1, H2, and H3 mutations and the CH<sub>2</sub> deletion (PCT Application No. 95/305444, published March 6, 1996)).

15

Figure 5 is a schematic diagram of a plasmid, designated pD17-cJ-dCH2.H1, of cBR96-A (SEQ ID NO. 10) (i.e., chimeric BR96 having the CH<sub>2</sub> deletion (PCT Application No. 95/305444, published March 6, 1996)).

20 Figure 6 is a schematic diagram of a plasmid, designated pD17-cJ.H1, of cBR96.

Figure 7 is a line graph showing the results of an ELISA assay of (1) hBR96-2A-Dox to Le<sup>y</sup> (closed diamond), (2) hBR96-2A to Le<sup>y</sup> (96:0006A2 R/A)(closed square), (3) hBR96-2A to Le<sup>y</sup> (96:0006B R/A)(closed triangle), and BR96-Dox to  
25 Le<sup>y</sup> (X).

Figure 8 is a line graph showing the results of an ELISA assay of (1) BR96-A-Dox to Le<sup>y</sup> (closed diamond), (2) chiBR96 to Le<sup>y</sup> (closed square), (3) cBR96-A to Le<sup>y</sup> (96:0003 R/A)(closed triangle), and cBR96-Dox to Le<sup>y</sup> (X).

Figures 9a-c are schematic diagrams showing the steps for deleting a CH<sub>2</sub> domain.

Figures 10a-c are schematic diagrams showing the construction of BR96 IgG1 CH<sub>2</sub>  
5 domain point mutations.

Figure 11 is a schematic diagram showing the construction of the pNg1/14 vector.

Figure 12 is a schematic diagram showing the construction of pD17-hBR96-2.

10

Figure 13 is a schematic diagram showing the construction of pD17-hJm14-  
dCH2.H1.

Figure 14 is the nucleic acid sequence of pD17-cJ-dCH2.H1, the plasmid shown in  
15 Figure 5, chimeric BR96 having the CH<sub>2</sub> deletion.

Figure 15 is a line graph showing the results of an ELISA assay comparing whole  
chiBR96 and deleted CH<sub>2</sub> chiBR96 on Le<sup>y</sup>.

20 Figure 16 is a description of the seven structural alterations.

Figure 17 is a schematic diagram of a plasmid designated pD17-hG1b.

Figure 18 is the nucleic acid sequence of pD17-hJm14.H1.

25

Figure 19 is the nucleic acid sequence of pD17-hG1b.

Figure 20 is a line graph showing complement dependent cytotoxicity. In the  
legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is

hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b mAb, negative control.

5 Figure 21 is a line graph showing antibody dependent cell-mediated cytotoxicity. In the legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b monoclonal antibody (mAb), negative control.

10

Figure 22 is a line graph showing binding activity of hBR96-2 constant region mutants on LeY-HSA. In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331  
15 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

Figure 23 is a line graph showing binding activity of hBR96-2 constant region mutants on LNFPIII-BSA. LNFPIII is a lacto-N-fucopentasose, a Lewis X  
20 trisaccharide with an additional lactose spacer (V Labs, Covington, LA). In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

25

Figures 24A and 24B provide a strategy for introducing multiple mutations by RPCR. (A) Diagram of the 1.4 kpb IgG heavy chain region showing the hinge CH<sub>2</sub> and CH<sub>3</sub> domains as boxed regions. Site-specific mutations to be introduced into CH<sub>2</sub> positions L1, L2, and L3 are encoded by complementary sets of mutant PCR

primers (A1 and A2; B1 and B2; and C1 and C2). The asterisks (\*) indicate the number of amino acid changes introduced at each L position. The two PCR primers, Rs (Recombination -sense) and Ra (Recombination-antisense), flank the Eco-47-III restriction sites and mediate homologous recombination with vector ends. The 3' ends of the oligonucleotides are represented by arrowheads. (B) A three-way homologous recombination event between fragments RsA2, A1Ra and the linearized vector produces the L1 mutant IgG. Two distally located sets of mutations (L1 and L2) are simultaneously introduced by increasing the number of recombining PCR produces as is shown in the four-way recombination of RsA2, A1B1, B1Ra with vector.

Figure 25 is a gel showing Eco-47-III restriction endonuclease analysis of DNAs prepared from colonies generated by multiple PCR fragment RPCR. Lane M: 1kb ladder DNA marker (GIBCO/BRL Life Science Technology). Lanes 1-12: Twelve randomly selected colonies resulting from quadruple homologous recombination events were used to prepare plasmid and digested with Eco47-III. Clones 1, 2, 6 and 9 contain the fully assembled 1.4 kpb insert.

Figure 26 provides the amino acid sequence for hBR96-2 heavy-chain variable region and the human IgG1 constant region.

Figure 27 provides the amino acid sequence for hBR96-2A heavy-chain variable region and the human IgG1 constant region.

Figure 28 provides the amino acid sequence for chi BR96 heavy-chain variable region and the human IgG1 constant region without the CH<sub>2</sub> domain.

## **DETAILED DESCRIPTION OF THE INVENTION**

### **DEFINITIONS**

5 As used herein the term "inhibiting immunoglobulin-induced toxicity" means to reduce or alleviate symptoms generally associated with toxicity caused by immunoglobulin or Ig fusion protein therapy, e.g., toxicity mediated by effector functions of the Fc receptor. For example, BR96 antibody recognizes and binds BR96 antigen which is found at some levels in the gastrointestinal tract and at  
10 elevated levels in tumors (as compared to the gastrointestinal tract of normal tissues). The binding of BR96 antibody to BR96 antigen in vivo causes symptoms associated with gastrointestinal toxicity. These symptoms include rapid onset of vomiting, often with blood, and nausea. In humans the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach.

15

The pathology of the wound is limited and resolves. However, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity. For highly elevated levels of other antigens found in the central nervous system (CNS), liver, and other locations, the toxicity will be characterized by  
20 symptoms other than those described above.

As used herein the term "immunoglobulin molecule" can be produced by B cells or be generated through recombinant engineering or chemical synthetic means. Examples of immunoglobulin molecules include (1) antibodies, e.g., polyclonal and  
25 monoclonal antibodies, chimeric or humanized, and (2) recombinant Ig containing binding proteins, e.g., Ig fusion proteins. Recombinant Ig containing binding proteins include cell surface proteins, e.g., CD antigens (in one embodiment, CTLA4), to which an Ig tail is joined.

As used herein the terms "structurally altered" or "structural alteration" means manipulating the constant region so that the resulting molecule or protein exhibits a diminished ability to induce toxicity. Structural alteration can be by chemical modification, proteolytic alteration, or by recombinant genetic means. Recombinant  
5 genetic means may include, but is not limited to, the deletion, insertion and substitution of amino acid moieties.

As used herein the terms "multiple toxicity associated domains" means more than one discrete toxicity associated domain. As there appear to be at least two toxicity  
10 associated domains in the immunoglobulin molecule, one roughly localized to amino acids 231-238 and another roughly localized to amino acids 310-331, an example of the structural alteration of multiple toxicity associated domains comprises the insertion, substitution or deletion of amino acid residues in both of these domains. This definition excludes structural alterations targeting a single toxicity associated  
15 domain.

Merely by way of example, the constant region of the immunoglobulin molecule can be structurally altered so that the molecule no longer mediates a CDC or ADCC response. However, the methods of the invention encompasses the use of  
20 structurally altered immunoglobulin molecules regardless of whether it mediates a CDC or ADCC response. The underlying requirement is that the altered molecule must inhibit immunoglobulin-induced toxicity.

Structural alteration can be effected in a number of ways. For example, structural  
25 alteration can be effected by deletion of the entire constant region.

Alternatively, structural alteration can be effected by deletion of the entire CH<sub>2</sub> domain of the constant region. In this instance, deletion of the entire CH<sub>2</sub> domain may render the molecule unable to (1) bind an Fc receptor thereby eliminating the



molecule's possibility of mediating antibody-dependent cellular cytotoxicity (ADCC), (2) bind C1q, or (3) activate complement.

Alternatively, structural alteration can be effected by deletion of only that portion of  
5 the CH<sub>2</sub> domain that binds the Fc receptor or complement.

Further alternatively, a single mutation or multiple mutations such as substitutions and insertions in the CH<sub>2</sub> domain can be made. The underlying requirement of any mutation is that it must inhibit, diminish, or block immunoglobulin-induced toxicity.  
10 For example, this can be achieved by mutating the constant region such that the altered molecule is rendered unable to mediate a CDC response or an ADCC response, or to activate complement.

Alternatively, structural alteration can be effected by isotype switching (also known  
15 as class switching) so that the altered molecule does not induce toxicity in the subject. In one embodiment, the constant region of the immunoglobulin is structurally altered so that it no longer binds the Fc receptor or a complement component, e.g., switching a molecule's original IgG isotype from IgG1 to IgG4. Isotype switching can be effected regardless of species, i.e., an isotype from a non-  
20 human being can be switched with an isotype from a human being (E.D. Finkelman et al. (1990) *Annu. Rev. Immunol.* 8:303-333; T. Honjo et al. (1979) *Cell* 18: 559-568; T. Honjo et al. In "Immunoglobulin Genes" pp. 124-149 Academic Press, London)).

25 As used herein the term "Ig fusion protein" means any recombinantly produced antigen or ligand binding domain having a constant region which can be structurally altered.

As used herein "cytotoxic agent" includes antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents. Specific examples within these groups include but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, 5 tenoposide, vincristine, vinblastine, colchicine, supporin, gelonin, PE40, bryodin, dihydroxy anthracin dione, actinomycin D, and 1-dehydrotestosterone.

As used herein the term "BR96" refers to (1) the whole BR96 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, (2) chimeric BR96 10 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, or (3) BR96 mutant molecules disclosed in PCT No. 95/305444, published March 6, 1996.

As used herein, "treating" means to (1) provide tumor regression so that the tumor is 15 not palpable for a period of time (standard tumor measurement procedures may be followed (A.B. Miller et al. "Reporting results of cancer treatment" Cancer 47:207-214 (1981)); (2) stabilize the disease; or (3) provide any clinically beneficial effects.

As used herein, an "effective amount" is an amount of the antibody, 20 immunoconjugate, or recombinant molecule which kills cells or inhibits the proliferation thereof.

As used herein, "administering" means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular or 25 subcutaneous administration, or the implantation of a slow-release device such as a miniosmotic pump, to the subject.

As used herein, "pharmaceutically acceptable carrier" includes any material which when combined with the antibody retains the antibody's specificity or efficacy and is

non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including  
5 coated tablets and capsules.

Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may  
10 also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods.

As used herein, "mutation" means a single amino acid or nucleic acid mutation or multiple mutations by whatever means, e.g., homologous recombination, error prone  
15 PCR, or site directed mutagenesis.

In order that the invention herein described may be more fully understood, the following description is set forth.

## 20 METHODS OF THE PRESENT INVENTION

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from the use of immunoglobulin during therapy or in vivo diagnosis. For example, the methods of the invention would be useful to minimize  
25 the toxicity associated with prolonged clinical exposure to immunoglobulin use during or after tumor imaging with radiolabeled antibodies.

In accordance with the practice of this invention, the subject includes, but is not limited to, human, equine, porcine, bovine, murine, canine, feline, and avian

subjects. Other warm blooded animals are also included in this invention.

This method comprises administering an immunoglobulin molecule to the subject. The immunoglobulin can be IgG, IgM, or IgA. IgG is preferred.

5

In one embodiment of the invention, the immunoglobulin molecule recognizes and binds Le<sup>y</sup>. In another embodiment, the immunoglobulin recognizes and binds Le<sup>x</sup>.

In a further embodiment, the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma deposited on February 22, 1989 with the American Type  
10 Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and  
accorded ATCC Accession No.: HB 10036. In yet another embodiment, the  
immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma  
deposited on May 23, 1990, with the ATCC, 12301 Parklawn Drive, Rockville, MD  
20852 and accorded ATCC Accession No.: HB 10460.

15

In accordance with the practice of the invention, the immunoglobulin can be a  
bispecific antibody with a binding specificity for two different antigens, one of the  
antigens being that with which the monoclonal antibody BR96 produced by the  
hybridoma having the identifying characteristics of HB 10036 as deposited with the  
20 ATCC binds. Also, in accordance with the practice of the invention, the  
immunoglobulin can be an anti-idiotypic antibody.

As required by the invention, at least a portion of the constant region of the  
immunoglobulin molecule is structurally altered. Structural alteration can be  
25 effected by a number of means. In one embodiment, the entire constant region, i.e.,  
CH<sub>1</sub>, CH<sub>2</sub>, and CH<sub>3</sub> domains, can be deleted.

In another embodiment, only the CH<sub>2</sub> domain is deleted from the immunoglobulin  
molecule (e.g., cBR96-A (Figure 5), hBR96-2A (Figure 4). In this embodiment, the

CH<sub>2</sub> deletion may result in a molecule unable to bind the Fc receptor or a complement component.

In another embodiment, only that portion of the CH<sub>2</sub> domain which binds the complement component C1q is deleted. In yet another embodiment, mutations in specific portions of the CH<sub>2</sub> domain are made. For example, the immunoglobulin molecule may be modified by structurally altering multiple toxicity associated domains in the constant region so that immunoglobulin-induced toxicity is inhibited. A discussion of such mutations are further found hereinafter.

10

Regardless of the means, the underlying requirement for any structural alteration of the constant region is that immunoglobulin-induced toxicity is substantially reduced or inhibited. In one embodiment, immunoglobulin-induced toxicity is inhibited by structurally altering the constant region such that the molecule's ability to mediate a CDC response or ADCC response and/or activate the complement cascade is prevented or inhibited. Methods for determining whether the molecule is able to inhibit a CDC response are well known, e.g., one method involves a <sup>51</sup>Cr-release test (H. Garrigues et al. Int. J. Cancer 29:511 (1982); I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to inhibit an ADCC response are well known (I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to activate a complement cascade are well known.

15

In another embodiment of the invention, the method comprises administering to the subject an Ig fusion protein having a structurally altered constant region. Structural alteration of the constant region may include deletion of the entire C region or portions thereof, e.g., alteration of the CH<sub>2</sub> domain so that the altered molecule no longer binds the Fc receptor or a complement component.

20

The invention further provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject. The method comprises administering to the subject an antibody which has been modified so that at least a portion of the constant region has been structurally altered as discussed supra. In one  
5 embodiment, the antibody recognizes and binds Le<sup>y</sup>. In another embodiment, the antibody recognizes and binds to Le<sup>x</sup>.

In accordance with the practice of this invention, the antibody can be monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of  
10 HB 10036 as deposited with the ATCC. Alternatively, the antibody can be chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC. Further, the antibody can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma  
15 having the identifying characteristics of HB 10036 as deposited with the ATCC binds.

Additionally, the present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a  
20 subject. The disease will vary with the antigen sought to be bound. Examples of diseases include but are not limited to immunological diseases, cancer, cardiovascular diseases, neurological diseases, dermatological diseases or kidney disease.

25 This method comprises the following steps. Step one provides selecting an antibody for a target. Generally, the target is associated with the disease and the antibody directed to the target is known. For example, the target can be the BR96 antigen and the antibody selected is BR96.

Step two of this method provides structurally altering the constant region of the antibody so selected so that immunoglobulin induced toxicity is inhibited. Inactivation can include any of the means discussed above. For example, inactivation can be effected by structurally altering multiple toxicity associated domains in the CH<sub>2</sub> domain of the constant region of the Ig protein so selected.

Step three of this method provides administering the structurally altered antibody of step two to the subject under conditions that the structurally altered antibody recognizes and binds the target and that such binding directly or indirectly alleviates symptoms associated with the disease.

In accordance with the invention, in one embodiment step one provides selecting an Ig fusion protein for a target. Further, the method provides mutating the Ig fusion protein so selected by structurally altering the CH<sub>2</sub> domain of the constant region of the Ig protein by the same means discussed above.

The invention further provides methods to treat human carcinoma. For example, the immunoglobulin, antibody, or Ig fusion protein discussed above can be used in combination with standard or conventional treatment methods such as chemotherapy, radiation therapy or can be conjugated or linked to a therapeutic drug, or toxin, as well as to a lymphokine or a tumor-inhibitory growth factor, for delivery of the therapeutic agent to the site of the carcinoma.

Techniques for conjugating therapeutic agents to immunoglobulins are well known (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellström et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In

Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)).

5

Alternatively, the structurally altered antibody or Ig fusion protein can be coupled to high-energy radiative agents, e.g., a radioisotope such as <sup>131</sup>I; which, when localized at the tumor site, results in a killing of several cell diameters (see, e.g., Order, "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985)). According to yet another embodiment, the structurally altered BR96 antibody can be conjugated to a second antibody to form an antibody heteroconjugate for the treatment of tumor cells as described by Segal in United States Patent 4,676,980.

15

Still other therapeutic applications for the structurally altered antibody or Ig fusion protein of the invention include conjugation or linkage, e.g., by recombinant DNA techniques or protein chemical techniques, to an enzyme capable of converting a prodrug into a cytotoxic drug and the use of that antibody-enzyme conjugate in combination with the prodrug to convert the prodrug to a cytotoxic agent at the tumor site (see, e.g., Senter et al., "Anti-Tumor Effects Of Antibody-alkaline Phosphatase", Proc. Natl. Acad. Sci. USA, 85:4842-46 (1988); "Enhancement of the in vitro and in vivo Antitumor Activities of Phosphorylated Mitomycin C and Etoposide Derivatives by Monoclonal Antibody-Alkaline Phosphatase Conjugates", Cancer Research 49:5789-5792 (1989); and Senter, "Activation of Prodrugs by Antibody-Enzyme Conjugates: A New Approach to Cancer Therapy," FASEB J. 4:188-193 (1990)).

25



It is apparent therefore that the present invention encompasses pharmaceutical compositions including immunoglobulin molecules, antibodies, and Ig fusion proteins all having structurally altered CH<sub>2</sub> domains, and their use in methods for treating human carcinomas. For example, the invention includes pharmaceutical  
5 compositions for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of a structurally altered BR96 and a pharmaceutically acceptable carrier.

The compositions may contain the structurally altered antibody or Ig fusion protein  
10 or antibody fragments, either unmodified, conjugated to a therapeutic agent (e.g., drug, toxin, enzyme or second antibody). The compositions may additionally include other antibodies or conjugates for treating carcinomas (e.g., an antibody cocktail).

The compositions of the invention can be administered using conventional modes of  
15 administration including, but not limited to, intrathecal, intravenous, intraperitoneal, oral, intralymphatic or administration directly into the tumor. Intravenous administration is preferred.

The composition of the invention can be in a variety of dosage forms which include,  
20 but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

25 The compositions of the invention also preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate.

In accordance with the practice of the invention, the pharmaceutical carrier can be a lipid carrier. The lipid carrier can be a phospholipid. Further, the lipid carrier can be a fatty acid. Also, the lipid carrier can be a detergent. As used herein, a detergent  
5 is any substance that alters the surface tension of a liquid, generally lowering it.

In one example of the invention, the detergent can be a nonionic detergent. Examples of nonionic detergents include, but are not limited to, polysorbate 80 (also known as Tween 80 or (polyoxyethylenesorbitan monooleate), Brij, and Triton (for  
10 example Triton WR-1339 and Triton A-20).

Alternatively, the detergent can be an ionic detergent. An example of an ionic detergent includes, but is not limited to, alkyltrimethylammonium bromide.

15 Additionally, in accordance with the invention, the lipid carrier can be a liposome. As used in this application, a "liposome" is any membrane bound vesicle which contains any molecules of the invention or combinations thereof.

The most effective mode of administration and dosage regimen for the compositions  
20 of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician.

The interrelationship of dosages for animals of various sizes and species and humans based on  $\text{mg/m}^2$  of surface area is described by Freireich, E.J., et al. Cancer  
25 Chemother., Rep. 50 (4): 219-244 (1966). Adjustments in the dosage regimen can be made to optimize the tumor cell growth inhibiting and killing response, e.g., doses can be divided and administered on a daily basis or the dose reduced proportionally depending upon the situation (e.g., several divided doses can be

administered daily or proportionally reduced depending on the specific therapeutic situation).

## THE MOLECULES OF THE INVENTION

5

The present invention provides structurally altered BR96 or BR96 Ig fusion proteins.

Structurally altered BR96 antibodies or Ig fusion proteins have the variable region of BR96 and a modified constant region. This modification provides structurally altered BR96 antibodies or Ig fusion proteins with the ability to inhibit  
10 immunoglobulin-induced toxicity.

Various embodiments of structurally altered BR96 or BR96 Ig fusion proteins have been made.

15 In one embodiment, designated cBR96-A, the entire CH<sub>2</sub> domain of cBR96 was deleted. CBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 10. cBR96 is expressed by a plasmid having the sequence in SEQ ID NO. 9.

20 In another embodiment, designated hBR96-2A, the entire CH<sub>2</sub> domain of hBR96 was deleted. hBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 12. hBR96 is a mutant BR96 having the H1, H2, and H3 mutations described in PCT Application No. 95/305444, published March 6, 1996.

25 In yet another embodiment, designated hBR96-2B, the leucine residue located at amino acid position 235 is mutated to alanine. Additionally, the glycine residue located at amino acid position 237 is mutated to alanine. The amino acid position numbering used is described in Kabat et al. Sequences of Proteins of Immunological Interest 5th Edition (1991) United States Department of Health and Human Services.

In a further embodiment, designated hBR96-2C, the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine  
5 using standard protocols (Alexander R. Duncan and Greg Winter "The binding site for C1q on IgG" Nature 332:738 (1988)).

In another embodiment, designated hBR96-2D, the proline residue at position 331 is mutated to alanine (M-H. Tao et al., "Structural features of human immunoglobulin  
10 G that determine isotype-specific differences in complement activation" J. Exp. Med. 178:661-667 (1993); Y. Xu et al., "Residue at position 331 in the IgG1 and IgG4 domains contributes to their differential ability to bind and activate complement" J. Biol. Chem. 269:3469-3474 (1994)).

15 In an additional embodiment, designated hBR96-2E, the leucine residue at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine (A. Morgan et al., "The N-terminal end  
20 of the CH<sub>2</sub> domain of chimeric human IgG1 anti-HLA-DR is necessary for C1q, Fc(gamma)RI and Fc(gamma)RIII binding" Immunol. 86:319-324 (1995)).

In yet a further embodiment, designated hBR96-2F, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is  
25 mutated to alanine; and the proline residue located at position 331 is mutated to alanine.

In yet another embodiment, designated hBR96-2G, the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is

mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

5 In another embodiment, designated hBR96-2H, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

10

Depending on its form, a structurally altered BR96 antibody or fusion protein can be a monofunctional antibody, such as a monoclonal antibody, or bifunctional antibody, such as a bispecific antibody or a heteroantibody. The uses of structurally altered BR96, i.e., as a therapeutic or diagnostic agent, will determine the different forms of  
15 structurally altered BR96 which is made.

Several options exists for antibody expression. Immunoexpression libraries can be combined with transfectoma technology, i.e., the genes for the Fab molecules derived from the immunoglobulin gene expression library can be connected to the  
20 desired constant-domain exons. These recombinant genes can then be transfected and expressed in a transfectoma that would secrete an antibody molecule.

Once produced, the polypeptides of the invention can be modified, i.e., by amino acid modifications within the molecule, so as to produce derivative molecules. Such  
25 derivative molecules would retain the functional property of the polypeptide, namely, the molecule having such substitutions will still permit the binding of the polypeptide to the BR96 antigen or portions thereof.

It is a well-established principle of protein chemistry that certain amino acid

substitutions, entitled "conservative amino acid substitutions," can frequently be made in a protein without altering either the conformation or the function of the protein.

- 5 Amino acid substitutions include, but are not necessarily limited to, amino acid substitutions known in the art as "conservative".

Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid  
10 (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa.

Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional  
15 structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine and valine (V).

Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R)  
20 are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

- 25 In one embodiment of the present invention, the polypeptide is substantially pure, i.e., free of other amino acid residues which would inhibit or diminish binding of the polypeptide to its target and would inhibit or reduce gastrointestinal toxicity which are normally exhibited during or after antibody therapy.

**NUCLEIC ACID MOLECULES ENCODING THE PRESENT INVENTION**

The nucleotide sequences and the amino acid sequences of the variable and constant regions of BR96 are known. The sequence for the immunoglobulin constant region  
5 is known and provided in Figure 18. Specific mutations in the constant region of the BR96 antibody were made. Nucleic acid molecules encoding the seven mutants described above (hBR96-2B through hBR96-2H) are as follows.

In hBR96-2B, alanine at amino acid positions 235 and 237 is encoded by codons  
10 GCU, GCC, GCA, or GCG.

In hBR96-2C, serine at positions 318, 320, and 322 is encoded by UCU, UCC, UCA, or UGG.

15 In hBR96-2D, alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2E, alanine at positions 235 and 237 is encoded by codons GCU, GCC, GCA, or GCG. Serine at positions 318, 320, and 322 is encoded by UCU, UCC,  
20 UCA, or UGG.

In hBR96-2F, alanine at positions 235, 237, and 331 is encoded by codons GCU, GCC, GCA, or GCG.

25 In hBR96-2G, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG. Further, the alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2H, alanine at positions 235, 237, and 331 is encoded by codons GCU,

GCC, GCA, or GCG. Additionally, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG.

Any of the above can be deoxyribonucleic acid (DNA), e.g., complementary DNA  
5 (cDNA), or ribonucleic acid (RNA).

## IMMUNOCONJUGATES

Immunoconjugates (having whole antibody or Ig fusion proteins) may be  
10 constructed using a wide variety of chemotherapeutic agents such as folic acid and anthracyclines (Peterson et al., "Transport And Storage Of Anthracyclines In Experimental Systems And Human Leukemia", in Anthracycline Antibiotics In Cancer Therapy, Muggia et al. (Eds.), p. 132 (Martinus Nijhoff Publishers (1982); Smyth et al., "Specific Targeting of Chlorambucil to Tumors With the Use of  
15 Monoclonal Antibodies", J. Natl. Cancer Inst., 76:503-510 (1986)), including doxorubicin (DOX) (Yang and Reisfeld "Doxorubicin Conjugated with a Monoclonal Antibody Directed to a Human Melanoma-Associated Proteoglycan Suppresses Growth of Established Tumor xenografts in Nude Mice PNAS (USA)" 85:1189-1193 (1988)), Daunomycin (Arnon and Sela "In Vitro and in vivo Efficacy  
20 of Conjugates of Daunomycin With Anti-Tumor Antibodies" Immunol. Rev., 65:5-27 (1982)), and morpholinodoxorubicin (Mueller et al., "Antibody Conjugates With Morpholinodoxorubicin and Acid-Cleavable Linkers", Bioconjugate Chem., 1:325-330 (1990)).

25 BR96 has been conjugated to doxorubicin and has been shown to be effective in therapy of certain cancers or carcinomas (Trail, P.A., Willner, D., Lasch, S.J., Henderson, A.J., Casazza, A.M., Firestone, R.A., Hellström, I., and Hellström, K.E. Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. Science, 261:212-215, 1993).



In accordance with the practice of the invention, structurally altered BR96 can be used in forms including unreduced IgG, reduced structurally altered IgG, and fusion proteins (PCT Application No. 95/305444, published March 6, 1996).

5

Suitable therapeutic agents for use in making the immunoconjugate includes Pseudomonas exotoxin A (PE) in either the native PE or LysPE40 form. LysPE40 is a truncated form containing a genetically modified amino terminus that includes a lysine residue for conjugation purposes. Doxorubicin is also a suitable therapeutic

10 agent.

Additional examples of therapeutic agents include, but are not limited to, antimetabolites, alkylating agents, anthracyclines, antibiotics, and anti-mitotic agents.

15

Antimetabolites include methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine.

Alkylating agents include mechlorethamine, thiotepa chlorambucil, melphalan, 20 carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin.

25 Anthracyclines include daunorubicin (formerly daunomycin) and doxorubicin (also referred to herein as adriamycin). Additional examples include mitozantrone and bisantrene.

Antibiotics include dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC).

Antimitotic agents include vincristine and vinblastine (which are commonly referred to as vinca alkaloids).

- 5 Other cytotoxic agents include procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons.

Further examples of cytotoxic agents include, but are not limited to, ricin, bryodin, gelonin, supporin, doxorubicin, taxol, cytochalasin B, gramicidin D, ethidium

- 10 bromide, etoposide, tenoposide, colchicine, dihydroxy anthracin dione, 1-dehydrotestosterone, and glucocorticoid.

Clearly analogs and homologs of such therapeutic and cytotoxic agents are encompassed by the present invention. For example, the chemotherapeutic agent

- 15 aminopterin has a correlative improved analog namely methotrexate.

Further, the improved analog of doxorubicin is an Fe-chelate. Also, the improved analog for 1-methylnitrosourea is lomustine. Further, the improved analog of vinblastine is vincristine. Also, the improved analog of mechlorethamine is

- 20 cyclophosphamide.

## METHODS FOR MAKING MOLECULES OF THE INVENTION

There are multiple approaches to making site specific mutations in the CH<sub>2</sub> domain of an immunoglobulin molecule. One approach entails PCR amplification of the

25 CH<sub>2</sub> domain with the mutations followed by homologous recombination of the mutated CH<sub>2</sub> into the vector containing the desired immunoglobulin, e.g., hBR96-2. For example, hBR96-2B and hBR96-2D have been made by this method.

Another approach would be to introduce mutations by site-directed mutagenesis of single-stranded DNA. For example, vector pD17-hG1b, which contains only the constant region of IgG1 and not the V domain of hBR96, has the fl origin of replication. This gives the vector the properties of a phagemid and site-directed  
5 mutagenesis experiments can be performed according to the methods of Kunkel, et al. (Kunkel, T.A., J.D. Roberts, and R.A. Zakour, 1987 Methods Enzymol. 154:367-383) as provided in the Bio-Rad Muta-Gene® phagemid *in vitro* mutagenesis kit, version 2. For example, hBR96-2B, -C, -D, -E, -F, -G, and -H were made by this method.

10

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the scope of this invention in any manner.

15

#### EXAMPLE 1

The following standard ELISA protocol was used.

- 20 **Materials:** Immulon2 96 well plates and Genetic Systems Specimen Diluent Concentrate (10x); antibody conjugate was Goat Anti Human Kappa-HRP Mouse Adsorbed, Southern Biotech. at 1:10,000 in Genetic Systems Conjugate Diluent (1x); Genetic Systems EIA Chromogen Reagent (TMB) (1:100); Genetic Systems EIA Buffered Substrate (1x); primary antibody or antigen were AffiniPure F(ab')<sub>2</sub>  
25 Fragment Goat Anti Human IgG Fc Fragment specific (Jackson Immuno Research), Goat Anti Human Kappa-UNLB (Southern Biotechnology Associates), Le<sup>y</sup>-HSA (Alberta Research Council).

**Methods:** Dilute primary antibody or antigen to 1.0 µg/ml in 0.05M Carb/Bicarb buffer. Add 100µl of the diluted solution per well in Immulon 2 plates. Seal plates and incubate O.N. at 4°C.

- 5 Block plates by flicking them and blotting on paper towels. Add 200µl/well of Genetic Systems, Specimen Diluent Concentrate (1x). Incubate at least 1 hour at room temperature and then dump the contents of the plates. Wash the plates 3x in saline/Tween. Blot to dry. Allow the plates to dry at R.T. (45 min. to 1 hour). Seal and store the plates at 4°C.

10

Test samples as follows. Dilute samples and standards in Specimen Diluent at 1:10. Perform serial dilutions in separate round bottom plates. Transfer 100µl/well of final dilutions to antigen coated assay plates; then incubate O.N. at 4°C. Wash plates 3x with saline/Tween.

15

For conjugation add 100 µl/well of antibody-HRP conjugate in Genetic Systems Conjugate Diluent (1x). Incubate plates at Room Temp. for 60 min. Wash plates 3x in saline/Tween.

- 20 Add 100 µl/well of Genetic Systems EIA Chromogen Reagent (TMB) 1:100 in EIA Buffered Substrate (1x). Incubate at R.T. for 15 min. and stop with 1N H<sub>2</sub>SO<sub>4</sub> 100 µl/well. Read plate at 450/630nm in EIA plate reader.

## EXAMPLE 2

25

Construction of CH<sub>2</sub> deleted BR96 molecules

**Strategy for Deleting CH<sub>2</sub> Domains:** To construct CH<sub>2</sub> deleted BR96 molecules, the hinge, CH<sub>2</sub> and CH<sub>3</sub> domains were removed from chimeric BR96 and humanized

BR9696-2 IgG1 molecules by an Eco47-III restriction digestion in non-coding regions. The hinge and CH<sub>3</sub> domains were amplified by polymerase chain reaction (PCR) from a human IgG1 (pNy1.14) molecule lacking the CH<sub>2</sub> domain. Two oligonucleotides (Sense 49mer, Antisense 50mer) homologous to the sequences of  
 5 IgG1 constant region at both sides preserving Eco47-III sites were synthesized. The amplified hinge and CH<sub>3</sub> domain PCR fragments were added into Eco47-III sites on BR96 IgG1 molecules by in vivo homologous recombination (P. Bubeck et al., Nucleic Acid Research (1993) 21:3601-3602). The new BR96 IgG1 molecules were verified by restriction mapping and sequencing.

10

A sewing PCR strategy was used for the construction of CH<sub>2</sub> deleted human IgG1 (pNy1.14) (Robert M. Horton, et al. (1990) Biotech 8 (5)P, 528).

The CH<sub>1</sub> domain was amplified as a 580 bp fragment with a sense oligonucleotide  
 15 (5' TGG CAC CGA **AAG CTT TCT GGG GCA GGC CAG GCC TGA** 3') (primer A) and an antisense oligonucleotide (5' **TCC GAG CAT GTT GGT ACC CAC GTG GTG GTC GAC GCT GAG CCT GGC TTC GAG CAG ACA** 3') (primer B) from a linearized human IgG1 constant region vector (pNy1.7). The PCR fragment extends from the 5' end of the Hind-III site (in bold) through the Cel-II, Sal-I, Dra-  
 20 III, Kpn-I, 6 bp nucleotide spacer and Mro-I sites (in bold) at the 3' end of the CH<sub>1</sub> domain.

The CH<sub>3</sub> domain was then partially amplified (to the Xba-I site) with a sense primer (5' **GTC GAC CAC CAC GTG GGT ACC AAC ATG TCC GGA GCC ACA**  
 25 **TGG ACA GAG GCC GGC T** 3') (primer C) and an antisense primer (5' **CTG GTT CTT GTT CAT CTC CTC TCT AGA TGG** 3') (primer D) from a linearized human IgG1 constant region vector (pNy1.7). A PCR fragment (about 150 bp) with Sal-I, Dra-III, Kpn-I, 6 nucleotide spacer and Mro-I sites (in bold) on its 5' end, extends only through the Xba-I site (in bold) within the CH<sub>3</sub> domain.

The CH<sub>1</sub> and CH<sub>3</sub> partial PCR fragments were combined in a PCR without any primer. The reaction was run through two full cycles of denaturation and re-annealing to allow the fragments to combine at the homologous region at the 3' ends. Primers A and D (described above) were added to the reaction and the PCR cycle was completed. The polymerase extends the DNA with primer A and primer D, yielding a full-length (660 bp) PCR fragment. The newly extended PCR fragment is arranged from the 5' end to the 3' end in the following order: Hind-III - CH<sub>1</sub> - Cel-II - Sal-I - Dra-III - Kpn-I - 6 bp spacer - Mro-I - CH<sub>3</sub> partial - Xba-I.

10

The combined PCR fragment, with the CH<sub>1</sub> and partial CH<sub>3</sub> domains, was then cloned by a blunt end ligation into a Sma-I site on a pEMBL18 vector and the sequence was confirmed by dideoxy sequencing (Sanger et al. (1977) PNAS (USA) 74:5463-5466).

15

To transfer the CH<sub>1</sub> and partial CH<sub>3</sub> into a mammalian expression vector, both the pEMBL18 and pN $\gamma$ 1.7 vectors were digested with Hind-III and Xba-I. The Hind-III and Xba-I fragment was ligated into the same sites on a linearized pN $\gamma$ 1.7 vector. The new construct, with CH<sub>1</sub> and a full CH<sub>3</sub> domain, was designated the pN $\gamma$ 1.10 vector.

20

The hinge fragment was amplified from a Hind-III digested pN $\gamma$ 1.7 vector with the primers designed to flank the hinge exon with a Sal-I and a Dra-III cloning site at each end. These sites also exist between the CH<sub>1</sub> and CH<sub>3</sub> domains of the pN $\gamma$ 1.10 construct. The sense oligonucleotide (5' ACC ATG **GTC GAC** CTC AGA CCT GCC AAG AGC CAT ATC 3') with a 6 bp spacer and a Sal-I cloning site (in bold) and the antisense oligonucleotide (5' CAT GGT **CAC GTG** GTG TGT CCC TGG ATG CAG GCT ACT CTA G 3') with a 6 bp spacer and a Dra-III cloning site (in bold) were used for the amplification of the hinge fragment (250 bp).

25

The hinge region PCR fragment was cloned into a Sma-I site on pEMBL18 by blunt end ligation. Both the pEMBL18 with the hinge domain and the pN $\gamma$ 1.10 with the CH<sub>2</sub> and CH<sub>3</sub> domains were digested with Sal-I and Dra-III. The digested hinge  
5 fragment was cloned into the Sal-I and Dra-III linearized sites on the pN $\gamma$ 1.10 vector. The new construct, now carrying the CH<sub>1</sub>, hinge and CH<sub>3</sub> domains, was designated pN $\gamma$ 1.11.

To make the final CH<sub>2</sub> deleted human IgG1 construct, both the pN $\gamma$ 1.11 construct  
10 and pN $\gamma$ 1.11 vector were digested with BamHI and HindIII. A fragment containing the CH<sub>1</sub>, hinge and CH<sub>3</sub> domains was cloned into the linearized pN $\gamma$ 1.11 vector. The new constant region IgG1 construct lacks the CH<sub>2</sub> domain and is designated pN $\gamma$ 1.14 (Figure 11).

15 For digestion of BR96 IgG1 with Eco47-III, a restriction fragment with hinge, CH<sub>2</sub> and CH<sub>3</sub> domains was identified on the constant region sequence of BR96 IgG1 vector in both chimeric and humanized molecules. The 5' end of this fragment lies inside the intron between CH<sub>1</sub> and hinge and the 3' end is located inside the CH<sub>3</sub> intron of the BR96 IgG1 molecule. The hinge, CH<sub>2</sub> and CH<sub>3</sub> domains (1.368 kb  
20 fragment) were removed from BR96 IgG1 molecules by Eco47-III restriction digestion. The Eco47-III is a blunt end cutter. The BR96 IgG1 DNA digested with this enzyme does not require any pretreatment before cloning. Figure 12 is a diagrammatic representation of the pD17-hBR96-2 vector showing the Eco47-III sites used in cloning.

25

The CH<sub>2</sub> deleted BR96 IgG1 was then constructed as follows. The hinge and CH<sub>3</sub> domains were amplified from a CH<sub>2</sub> deleted L6 IgG1 (pN $\gamma$ 1.14) construct with a sense oligonucleotide (5'  
CAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCGCTGACCTCAG

A 3') homologous to the constant region sequence of IgG1 at the 5' end of the Eco47-III site (in bold) and an antisense oligonucleotide

(5'GGAAAGAACCATCACAGTCTCGCAGGGG

CCCAGGGCAGCGCTGGGTGCTT 3') homologous to the constant region

5 sequence of IgG1 at the 3' end of the Eco47-III site (in bold). The Eco47-III site at the 3' end of the pN $\gamma$ 1.14 construct is modified in the cloning process. The Eco47-III site is thus introduced into an antisense primer and used in amplification of the hinge and CH<sub>3</sub> domains.

10 The pD17-BR96 IgG1 vector was digested with Eco47-III and the hinge, CH<sub>2</sub> and CH<sub>3</sub> domains were removed. The linearized pD17-BR96 IgG1 vector was mixed with equimolar amounts of hinge and CH<sub>3</sub> PCR fragments. Cotransformation of the PCR fragment with linearized DNA into E.coli DH5a competent cells resulted in a recombinant molecule, mediated by homologous recombination in bacteria. This  
15 construct lacks the CH<sub>2</sub> domain of BR96 IgG1 molecules, and is designated pD17-BR96-dCH2 (Figure 13).

1.9 grams of CH<sub>2</sub>-deleted chimeric BR96 was obtained as raw material from 89L of culture supernatant.

20

### EXAMPLE 3

Toxicity, localization and clearance of CH<sub>2</sub>-deleted chimeric BR96 was tested in vivo as follows.

25

Three dogs received 400 mg/m<sup>2</sup> of cBR96-A, the CH<sub>2</sub> deletion mutant of chimeric BR96, and two received chimeric BR96. Both molecules had been mildly reduced and alkylated. This is required to prevent dimerization of the deletion mutant into a tetravalent form. Both control dogs experienced the typical GI toxicity and none of



the three receiving the mutant displayed any toxicity. The control dogs and two of the test dogs were sacrificed at 1 hr to obtain duodenal tissue to measure antibody localization. Both control dogs had grossly visible GI pathology, and the test dogs had normal appearing GI tissue. The third dog has continued to show no signs of toxicity.

**Results:** A significant amount of localization of the CH<sub>2</sub> deleted cBR96 (cBR96-A) occurred to the GI tract in dogs treated with 400 mg/m<sup>2</sup>, although the intact chiBR96 localized slightly better. The levels of localization indicate that roughly equivalent amounts of intact and CH<sub>2</sub> deleted cBR96 was delivered to the GI tract in these dogs.

Table 5. Localization of cBR96 to GI tissue.

Group	Animal	Specific	mean
		Localization	
cBR96	#271	155	135
	#272	114	
cBR96-A	#273	126	89
	#274	52	

15

Using the mean level of specific localization, an amount of cBR96-A equivalent to at least 66% of the amount of cBR96 was delivered to the target organ of toxicity, the duodenum. Based on the dose ranging done with cBR96 in dogs (some clinical signs of toxicity seen at doses of 10 mg/m<sup>2</sup>), even if this difference is real, it could

20

not explain the difference between significant toxicity and no toxicity, evaluation to date indicated that dogs treated with cBR96-A had no toxicity, pending microscopic histopathologic examination. This evaluation was based on analysis of 2 frozen blocks per dog and 2 sections per block. Replicates were quite good. We also ran  
5 historical frozen tissues from dogs treated with native cBR96 or F(ab)2/BR96 and the levels of localization for those tissues were 110 and 0, respectively, consistent with our previous data.

Assuming that there is no toxicity at marginally higher (2X) doses of cBR96-A,  
10 these data indicate that the CH<sub>2</sub> domain is associated with the induction of acute gastroenteropathy, and that the removal of this domain prevents the induction of gastroenteropathy mediated by BR96.

This study confirms the results showing that F(ab')<sub>2</sub> is not toxic in the dog model  
15 and that the toxicity is mediated by the constant region. The CH<sub>2</sub> deletion mutant is a candidate for targeting agents clinically. Because of the very long half-life of chimeric BR96, some decrease in the mutant's half-life should be acceptable.

Figure 1 shows the measurement of the clearance of the cBR96-A in high Le<sup>Y</sup>  
20 expressing dogs. The study used chimeric versus constant region mutant of cBR96-2.

CBR96-2 did clear faster than the chimeric BR96. The localization of cBR96-A to the gastrointestinal epithelium is not significantly affected by this more rapid  
25 clearance. More than enough of the cBR96-A localized to have caused toxicity.

**Discussion:** The constant region of chimeric IgG is responsible for the GI toxicity seen in clinical trials, e.g. with chiBR96-dox. The GI toxicity seen in the dog model is very similar to the clinical toxicity. Both in man and dog, administration of the

unconjugated antibody mediates an acute GI toxicity characterized by rapid onset of vomiting, often with blood.

5 In man the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach. Although the pathology of the wound is limited and resolves, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity.

10 This toxicity is mediated in man and dog by the antibody molecule alone. At higher doses of the antibody-dox conjugate, additional toxicity is seen in the dog model, probably due to doxorubicin. Although the intact IgG of BR96 causes toxicity in dog and man, the F(ab')<sub>2</sub> molecule (divalent and lacking only in the constant region) is not toxic in dogs. This finding has motivated our attempts at high levels, and improves the affinity and specificity of BR96 for tumor antigen.

15

The CH<sub>2</sub> domain is known to mediate complement and FcR binding. It was not known that structural alteration of the CH<sub>2</sub> domain would result in immunoglobulin-induced toxicity inhibition.

#### 20 Toxicology study of hBR96-2B

The toxicology study of hBR96-2B in high Lewis Y expressor dogs (n=2) showed that a dose of 400 mg/m<sup>2</sup> did not cause hematemesis nor bloody stools, in contrast to BR96 which consistently causes one or both signs. A dog sacrificed at 24 hrs had  
25 normal gross appearance of the GI tract, again in marked contrast to chimeric BR96 which causes hemorrhagic lesions and mucosal erosions.

**EXAMPLE 4**

- The polymerase chain reaction (PCR) is a widely used and versatile technique for the amplification and subsequent modification of immunoglobulin genes. The
- 5 rapidity and accuracy with which antibody genes can be modified in vitro has produced an assortment of novel antibody genes can be modified in vitro has produced an assortment of novel antibodies. For example, PCR methods have been used for engineering antibodies with increased affinity to antigen, for "humanizing" antibodies, and for modulating effector function (Marks, J.D., A.D. Griffiths, M.
- 10 Malmqvist, T. Clackson, J.M. Bye and G. Winter. 1992. Bypassing immunization: high affinity human antibodies by chain shuffling. *Bio/Technology* 10:779-783; Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96
- 15 Fab. *J. Biol. Chem.* 271:22611-22618; Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. *Immunology*. 86:319-324).
- 20 As part of a more comprehensive study, we desired to introduce various site specific mutations in the CH<sub>2</sub> constant domain of human IgG<sub>1</sub>. Six specific amino acid residues distributed throughout the CH2 domain previously identified to play a role in immune effector function were marked as targets for mutagenesis (Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-
- 25 terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. *Immunology*. 86:319-324; Duncan, A.R. and G. Winter. 1988. The binding site for Clq on IgG. *Nature* 332:738-740; Tao, M.-H., R.I.F. Smith and S.L. Morrison. 1993. Structural features of human immunoglobulin G that determine isotype-specific differences in complement

activation. J.Exp.Med. 178:661-667). five of the six residues were grouped into two clusters-one cluster consisting of two residues, two amino acids apart (Location 1, or L1); and a second cluster consisting of three residues spanning a sequence of five amino acids (L2). The remaining amino acid position (L3) made for the total of six  
5 residues. We were interested in constructing a panel of mutant CH<sub>2</sub> domain IgGs consisting of each L mutation by itself as well as in combination with other L mutants (e.g., L1; L1; and L2; L1, L2 and L3; etc.).

Various *in vitro* methods have been described where PCR is used to simultaneously  
10 introduce distally located site-specific mutations within a gene sequence (Ho, S.N., H.D. Hunt, R.M. Horton, J.K. Pullen and L.R. Pease. 1989. Site-directed mutagenesis by overlap extension. Gene 77:51-59; Ge, L. and P. Rudolph. 1996. Simultaneous introduction of multiple mutations using overlap extension PCR. BioTechniques 22:28-30). Alternatively, an *in vivo* procedure termed recombination  
15 PCR (RPCR) has also successfully been used for rapidly and efficiently generating distally located site-specific mutations (Jones, D.H. and S.C. Winistorfer. 1993. Use of polymerase chain reaction for making recombinant constructs. p.241-250. In B.A. White (Ed.), Methods in Molecular Biology, Vol. 15. Humana Press Inc., Totowa, NJ, Jones, D.H. And B.H. Howard. 1991. A rapid method for  
20 recombination and site-specific mutagenesis by placing homologous ends on DNA using polymerase chain reaction. BioTechniques 10:62-66). RPCR uses *E. Coli*'s recombination machinery to generate intact circular recombinant plasmids from a transfected mixture of linear PCR-generated product and linearized vector. *In vivo* recombination is mediated through the joining of nucleotide sequences designed into  
25 the 5' ends of both PCR primers that are homologous to DNA sequences encoded by the vector. In this report we describe an extension of the RPCR procedure for simultaneously introducing complex combinations of mutations into an antibody CH<sub>2</sub> domain.

- Humanized BR96 variable region heavy and light chain genes, previously cloned and co-expressed as an assembled active Fab fragment in an M13 phage expression vector, provided the starting material (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96 Fab. *J. Biol. Chem.* 271:22611-22618). The heavy and light chain V genes were amplified by PCR from a single-stranded M13 DNA template and subcloned by *in vivo* recombination (Jones, D.H. And B.H. Howard. 1991. A rapid method for recombination and site-specific mutagenesis by placing homologous ends on DNA using polymerase chain reaction. *BioTechniques* 10:62-66) into vectors pD17-hG1a and pD16-hCκ, to form pBR96-hG1a and pBR96-hCκ respectively. pD17-hG1a and pD16-hCκ are eukaryotic immunoglobulin expression vectors derived from pcDNA3 (Invitrogen, San Diego, CA). The plasmid pBR96-hG1a was further modified by site-directed mutagenesis to introduce two Eco47-III restriction sites flanking the immunoglobulin hinge-CH<sub>2</sub>-CH<sub>3</sub> domains using standard procedures. The recipient vector was then prepared by digesting pBR96-hG1a with Eco47-III, isolating the vector backbone by agarose gel electrophoresis followed by extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA).
- The strategy for introducing multiple mutations within the immunoglobulin CH<sub>2</sub> gene, shown in Figure 24, relies on the *in vivo* homologous recombination of several independently amplified PCR products with each other as well as with the pBR96-hG1a vector DNA. For introducing mutations at two distal locations two PCR products are synthesized (Figure 24B). One end of each PCR product is for recombining with an homologous end of the linear vector, and the other end, encoding the mutation(s) of interest, is for recombining with the neighboring PCR product. As shown in Figure 24B, additional distally-located mutations can be introduced into a target sequence by increasing the number of PCR products

proportionately. The recombination of neighboring PCR products always occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends (e.g., A1, A2) contain complementary mutant residues.

The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers (Rs and Ra) contain sequences for recombining with the end regions of the Eco47-III digested pBR96-hG1a vector.

Each L mutation was amplified in a separate PCR reaction. The reaction conditions were 250 ng intact pBR96-hG1a DNA template, 10 ul of 1X *Pfu* buffer (Stratagene, Inc. San Diego, CA), 10 nmol dNTPs, 200ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase in a 100ul reaction volume. Samples were first denatured at 95° C for 5 min, cooled to 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, followed by a final extension at 72°C for 7 min in a Perkin-Elmer DNA Thermal Cycler (Norwalk, CT). The amplified products were purified from a 1% agarose gel, extracted with Qiagen Gel Extraction kit and the recovered DNA quantitated. 50 ng of each PCR product was mixed with 25 ng of the Eco47-III digested pBR96-hG1a vector, transfected into Max competent *E. coli* DH5α according to the manufacturer's procedure (GIBCO BRL/Life Technologies, Gaithersburg, MD), and the entire transfection reaction plated onto selective LB agar plates containing 100 ug/ml ampicillin.

The results of several cloning experiments are summarized in the Table that follows. Typically the transformations produced from 80 to 200 bacterial colonies. Individual colonies were selected and grown overnight in 2 ml liquid cultures for isolation of miniprep plasmid DNA (Qiagen) and analysis by Eco47-III restriction

endonuclease mapping. Among 24 independent transformants analyzed from triple homologous recombination events (two PCR products plus vector) 11 clones contained the predicted 1.4 kpb DNA insert.

- 5 Figure 25 shows a sample diagnostic restriction analysis of DNA prepared from clones derived from quadruple homologous recombination events (three PCR products plus vector). Additional sampling of clones resulting from quadruple recombination yielded a cloning efficiency of 29% (7 clones containing inserts/24 clones sampled). At this point, due to the small sampling sizes, we do not know  
10 whether the differences in the cloning efficiencies observed between the triple and quadruple recombination events are meaningful.

- To evaluate the expression of Le $\gamma$  -binding activity of the CH<sub>2</sub> mutant IgGs, miniprep DNAs from 6 clones derived from the triple recombination reaction and 6  
15 clones derived from the quadruple recombination reaction exhibiting the predicted diagnostic Eco47-III restriction patterns were isolated, mixed with pBR96- hCk DNA and used to co-transfect COS7 cells. 48 hour spent supernatants from 3 ml cultures were assayed for total IgG production and for Le $\gamma$  binding activity by enzyme-linked immunosorbent assay (EIA) as described (Yelton, D.E., M.J. Rosok,  
20 G.A. Cruz, W.L. Cosand, J. Bajorath, I. Hellstom, K.-E. Hellstorm, W.D. Huse and S.M. Glaser. 1995. Affinity maturation of the BR96 anti-carcinoma antibody by codon-based mutagenesis. J.Immunol. 155:1994-2004). All twelve cultures were found to secrete approximately 2-3 ug/ml Le $\gamma$  -reactive IgG. The spectrum of Le $\gamma$  binding activities were all similar to that of native humanized BR96 IgG indicating  
25 that the homologously recombined antibodies did not acquire any gross mutations that could affect antigen binding. To confirm that the desired CH<sub>2</sub> mutations had been incorporated, and to evaluate the recombined genes for misincorporated nucleotides, four of the clones producing functional antibody were sequenced using Sequenase Version 2 DNA Sequencing Kit (United States Biochemical). One clone



was found to contain a single nucleotide change within the forward PCR primer used for mediating recombination with vector DNA. We are uncertain whether this error occurred during chemical synthesis of the oligonucleotide primer or is a result of misincorporation during the PCR reaction, despite the fact that we used a

5 thermostable polymerase with proofreading activity.

A RPCR procedure for homologously recombining up to three separate PCR-generated mutated antibody sequence products into a eukaryotic expression vector for the rapid construction of engineered IgG molecules is described herein. The

10 advantage of this approach is the ability to simultaneously introduce multiple distally-located mutations with PCR products synthesized by a single round of PCR. Recombinant DNAs are produced with a reasonably high cloning efficiency and fidelity of correct nucleotide sequences. The ability to efficiently rejoin several distinct PCR products should permit combinatorial strategies for constructing

15 complexly mutated protein domains as well as broadening the number and location of desired mutations.

Analysis of transformants generated by multiple-fragment RPCR.

Mutant IgGs Constructed	PCR Fragments in reaction	HR <sup>a</sup> events	Colonies Analyzed	Cloning Efficiency <sup>b</sup>
2	2	triple	24	45%
2	3	quadruple	24	33%
<sup>a</sup> HR-homologous recombination				
<sup>b</sup> Cloning efficiency (number of clones containing 1.4kbp insert/total number of colonies)				

**EXAMPLE 5**

This example provides two methods for introducing site specific mutations into the  
5 CH2 domain of human IgG1 constant region containing vectors.

One method involves PCR amplification of a segment or segments of the constant  
region, wherein mutations are introduced using appropriately constructed  
oligonucleotides. The vector receiving the fragment(s) is digested with a restriction  
10 enzyme to linearize the vector. PCR amplification primers are designed so that the  
5' ends of the PCR fragments can hybridize to the DNA sequence of the vectors. If  
more than one PCR fragment is amplified, then common sequences to the two  
fragments are introduced by oligonucleotides. Bacteria are transfected with the PCR  
fragments and with the digested vector. The fragments and vector can recombine by  
15 homologous recombination using the bacteria's recombination machinery. Bacterial  
colonies are selected and the DNA is analyzed by size and restriction map as a  
preliminary determination that the vector and fragment(s) recombined correctly.  
Correct insertion of fragments with the mutations is confirmed by dideoxynucleotide  
sequence analysis. DNA is then introduced into mammalian cells as described for  
20 the CH2 deleted antibody, and the expressed antibody analyzed for binding and  
functional activity.

By way of example, mutations Leu to Ala at residue 235 in CH2 and Gly to Ala at  
residue 237 were introduced by the procedure disclosed in Example 4. The heavy  
25 chain vector used for this procedure was pD17-hG1a, similar to pD17-BR96 vector  
described herein except that humanized V regions (Rosok, M.J., D.E. Yelton, L.J.  
Harris, J. Bajorath, K-E. Hellstrom, I, Hellstrom, G.A. Cruz, K. Kristensson, H. Lin,  
W.D. Huse, and S.M. Glaser, 1996. J. Biol. Chem 271 37:22611-22618) with three  
affinity mutations (H1, H2, and H3 mutations) were substituted.

pBR96-hG1a contains two *Eco*47-III restriction sites flanking the Ig hinge-CH2-CH3 domains. The recipient vector was prepared by (1) digesting pBR96-hG1a with *Eco*47-III, (2) isolating the vector by agarose gel electrophoresis, and (3)  
5 extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA). To introduce mutations at a single location, such as for positions 235 and 237, two PCR products were synthesized.

To introduce two distally located mutations, such as for mutant F (also referred to  
10 herein as hBR96-2F) with mutations at 235, 237, 331, requires 3 PCR products. The recombination of neighboring PCR products occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends contain complementary mutant residues. The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence flanking each side of the mutant residues for  
15 either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers containing sequences for recombining with the end regions of the *Eco*47-III digested pBR96-hG1a vector.

PCR amplification used 250 ng intact pBR96-hG1a DNA template, 10  $\mu$ l of 10X *Pfu*  
20 buffer (Stratagene, Inc., San Diego, CA), 10 nmol dNTPs, 200 ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase (Stratagen, Inc. San Diego, CA) in 100  $\mu$ l reaction. Samples were denatured at 95°C for 5 min, annealed at 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45  
25 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, and a final extension at 72°C for 7 min. The amplified products were purified from a 1% agarose gel, extracted with the Qiagen Gel Extraction kit and quantitated. 50 mg of each PCR product was mixed with 25 ng of the *Eco*47-III digested pBR96-hG1a vector and transfected in *E. coli* MAX Efficiency DH5 $\alpha$ <sup>TM</sup> according to the

manufacturer's instructions (GIBCO BRL/Life Technologies, Gaithersburg, MD).

The entire transfection reaction was plated onto LB agar plated containing 100 µg/ml ampicillin.

- 5 Bacterial colonies were selected and grown overnight at 37° C in 2 ml liquid cultures. DNA was isolated and analyzed by Eco47-III restriction endonuclease mapping. Clones with the correct size insert were sequenced (Sequenase Version 2, U.S. Biochemical Corp., Cleveland, OH).
- 10 The second method for introducing site specific mutations into the CH<sub>2</sub> domain of human IgG1 involved the method of Kunkel (1987 Methods Enzymology, supra). For this procedure pD17-hG1b DNA with the F1 origin of replication was introduced into electrocompetent E. coli CJ236 dut-ung- (Bio-Rad Laboratories, Hercules, CA) by electroporation according to manufacturer's instructions. PD17-
- 15 hG1b is a vector having a constant region but no variable region. The F1 ori site allows treatment of this vector as a phagemid.

Bacteria containing the plasmid were selected by ampicillin resistance. Single stranded uridynylated DNA was prepared using the Muta-Gene Phagemid In Vitro

- 20 Mutagenesis Version 2 protocol (Bio-Rad). Mutations were introduced by site-directed mutagenesis with the appropriate antisense oligonucleotide. For molecules with mutations at more than one location, mutations were introduced by either of the two methods discussed above. One method would be to (1) prepare one mutant, for example, mutant 2C (also referred to herein as BR96-2C) with the mutations at
- 25 residues 318, 320, 322, (2) isolate ssDNA, and (3) introduce a second mutation set with the appropriate anti-sense oligonucleotide. The second method would be to anneal two antisense oligonucleotides with the same uridynylated ssDNA and screen for mutants with both sets of changes. Mutant 2H (hBR96-2H) was also prepared by a combination of these methods.

The V region of humanized BR96-2 heavy chain was introduced by the homologous recombination method described above in pD17-hJm14.H1. The pD17-hJm14.H1 plasmid contains the BR96 humanized variable region with the H1/H2/H3

- 5 mutations and the plasmid was used to transfect mutant sequences into mammalian cells. The pD17G1b vector containing the Fc mutation(s) was digested with NheI for 3 hr at 37° C and the DNA isolated by methods described above. Insertion of the V region into the vector was determined by size and restriction enzyme mapping and confirmed by sequence analysis.

10

Transient expression of whole antibodies was performed by transfection of COS cells. For production of antibody, stable transfections of CHO cells were performed (see description of deleted CH2 mutant). All mutants were purified from CHO culture supernatants by protein A chromatography.

15

The oligonucleotide primers homologous to the vector and used to introduce the constant regions mutations were as follows:

Oligonucleotides homologous to vector sequences:

**Sens(sense)CH2 E47-3-5:** CAG GGA GGG AGG GTG TCT GCT GGA AGC

- 20 CAG GCT CAG CGC TGA CCT CAGA

**D CH2 E47-3 A (antisense):** GGA AAG AAC CAT CAC AGT CTC GCA GGG  
GCC CAG GGC AGC GCT GGG TGC TT

- 25 Oligonucleotides to mutate Leu235 to Ala and Gly237 to Ala (underlined sequences show sites of mutation):

**Antisense CH2 L235-G237/aa:** GAA GAG GAA GAC TGA CGG TGC CCC  
CGC GAG TTC AGG TGC TGA GG

**SensCH2 L235-G237/AA:** CCT CAG CAC CTG AAC TCG CGG GGG CAC  
CGT CAG TCT TCC TCT TC

Oligonucleotides to mutate Glu318, Lys320, Lys322 to Ser

**Antis(antisense)CH2 EKK/SSS-2:** CTG GGA GGG CTT TGT TGG AGA CCG  
ΔGC ACG ΔGT ACG ΔCT TGC CAT TCA GCC

5 Oligonucleotides to mutate Pro331 to Ala:

**Antis CH2 P331/A/3:** GAT GGT TTT CTC GAT GGC GGC TGG GAG GGC

**Sense CH2 P33/A:** GCC CTC CCA GCC GCC ATC GAG AAA ACC ATC

Alternative antisense oligo to introduce Ala at 331 by site-directed mutation:

**CH2P331A:** GAT GGT TTT CTC GAT AGC GGC TGG GAG GGC TTT G

10

Oligonucleotides to mutate Glu318 to Ser, Lys320 to Ser, Lys322 to Ser, and Pro331 to Ala:

**Antis CH2 EKKP/SSA-6:** GAT GGT TTT CTC GAT GGC GGC TGG GAG

GGC TTT GTT GGA GAC CGA GCA CGA GTA CGA CTT GCC ATT CAG

15 CCA GTC CTG GTG

**Sense CH2 EKKP/SSA-6:** CAC CAG GAC TGG CTG AAT GGC AAG TCG

TAC TCG TGC TCG GTC TCC AAC AAA GCC CTC CCA GCC GCC ATC

GAG AAA ACC ATC

20

#### In vitro Assays of the Mutants

Results of the CDC demonstrate that mutant hBR96-2B has approximately 10 fold less activity than the control hBR96-1 (two affinity mutations, one in H2 and one in  
 25 H3, refer to previous patent (Figure 20)). The mutants that have the least ability to kill cells in the presence of complement is hBR96-2C with the triple mutations at positions 318, 320, and 322 and the hBR96-2H mutant (least cytotoxic antibodies in the panel) which contains all six mutations at the three different locations. ADCC activity was most affected by the CH2 deleted hBR96-2 molecule (Figure 21).

hBR96-2B and -2H lost between 100 and 1000 fold activity to kill in the presence of effector cells. In the ADCC assay the hBR96-2B molecule also lost approximately 10 fold activity (Figure 21).

- 5    Figures 26-28 provide the amino acid sequences for the heavy chain variable region for both chimeric and humanized BR96 having the H1, H2, and H3 mutations. The amino acid sequence for the light chain variable region is known and methods for generating it are found in PCT Application No. 95/305444. Additionally provided is the amino acid sequence for the IgG1 constant region. Mutations in the constant
- 10    region are marked.

## SEQUENCE LISTING

- 5 (1) GENERAL INFORMATION
- (i) APPLICANT: Bristol-Myers Squibb Co.
- (ii) TITLE OF THE INVENTION:  
10 A METHOD FOR INHIBITING  
IMMUNOGLOBULIN-INDUCED TOXICITY FROM THE USE OF  
IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS
- (iii) NUMBER OF SEQUENCES: 13
- 15 (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: Merchant & Gould  
(B) STREET: 11150 Santa Monica Blvd., Suite 400  
(C) CITY: Los Angeles  
(D) STATE: CA  
20 (E) COUNTRY: USA  
(F) ZIP: 90025
- (v) COMPUTER READABLE FORM:  
25 (A) MEDIUM TYPE: Diskette  
(B) COMPUTER: IBM Compatible  
(C) OPERATING SYSTEM: DOS  
(D) SOFTWARE: FastSEQ Version 2.0
- (vi) CURRENT APPLICATION DATA:  
30 (A) APPLICATION NUMBER: PCT/US97/\_\_\_\_\_  
(B) FILING DATE: 01-AUG-1997  
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:  
35 (A) APPLICATION NUMBER: 60/023,033  
(B) FILING DATE: 02-AUG-1996
- 40 (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: Adriano, Sarah B  
(B) REGISTRATION NUMBER: 34,470  
(C) REFERENCE/DOCKET NUMBER: 30436.43WOU1
- 45 (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: 310-445-1140  
(B) TELEFAX: 310-445-9031  
(C) TELEX:
- 50 (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:  
55 (A) LENGTH: 36 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear



(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5 TGGCACCGAA AGCTTTCTGG GGCAGGCCAG GCCTGA 36

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 57 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCCGGACATG TTGGTACCCA CGTGGTGGTC GACGCTGAGC CTGGCTTCGA GCAGACA 57

20 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 55 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCCACCACC ACGTGGGTAC CAACATGTCC GGAGCCACAT GGACAGAGGC CGGCT 55

35 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTGGTTCTTG TTCATCTCCT CTCTAGATGG 30

(2) INFORMATION FOR SEQ ID NO:5:

50 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

55 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACCATGGTCG ACCTCAGACC TGCCAAGAGC CATATC 36

(2) INFORMATION FOR SEQ ID NO:6:

5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 39 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

15 CATGGTCACG TGGTGTGTCC CTGGATGCAG GCTACTCTA 39

(2) INFORMATION FOR SEQ ID NO:7:

20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 49 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

30 CAGGGAGGGA GGGTGTCTGC TGGAAGCCAG GCTCAGCGCT GACCTCAGA 49

(2) INFORMATION FOR SEQ ID NO:8:

35 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 50 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

45 GGAAAGAACC ATCACAGTCT CGCAGGGGCC CAGGGCAGCG CTGGGTGCTT 50

(2) INFORMATION FOR SEQ ID NO:9:

50 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8691 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCCG GCTTCGAATA GCCAGAGTAA 60  
CCTTTTTTTT TAATTTTATT TTATTTTATT TTTGAGATGG AGTTTGCGC CGATCTCCCG 120

	ATCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGCGC	GAGCAAAAT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGTTTTCGCG	300
	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAAT	360
5	AGTAATCAAT	TACGGGGTCA	TTAGTTCATA	GCCCATATAT	GGAGTTCCGC	GTTACATAAC	420
	TTACGGTAAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGTCAATAA	480
	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCT	TTGACGTCAA	TGGGTGGACT	540
	ATTTACGGTA	AACTGCCCAC	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCG	CCTGGCATT	TGCCCAGTAC	ATGACCTTAT	660
10	GGGACTTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	720
	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCTAA	840
	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAAGCCAC	TGCTTACTGG	CTTATCGAAA	960
15	TTAATACGAC	TCACTATAGG	GAGACCCAAG	CTTGGTACCA	ATTTAAATTG	ATATCTCCTT	1020
	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGCGGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCCT	TGTTTAAAAA	GGTGTCCAGT	1140
	GTGAAGTGAA	TCTGTGGGAG	TCTGGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
	TCTCTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATTA	CATGTATTGG	GTTTCGCCAGA	1260
20	CTCCAGAGAA	GAGGCTGGAG	TGGGTGCGAT	ACATTAGTCA	AGGTGGTGAT	ATAACCGACT	1320
	ATCCAGACAC	TGTAAAGGGT	CGATTACCCA	TCTCCAGAGA	CAATGCCAAG	AACACCCTGT	1380
	ACCTGCAAAT	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500
	CTAGACACAA	GGGCCCATCG	GTCTTCCCCC	TGGCACCCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
25	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCCTGT	ACGGTGTCTG	1620
	GGAACTCAGG	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCTTA	CAGTCTCAG	1680
	GACTCTACTC	CCTCAGCAGC	GTGGTCACCG	TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCT	1740
	ACATCTGCAA	CGTGAATCAC	AAGCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTTGGTGAGA	1800
	GGCCAGCACA	GGGAGGGAGG	GTGTCTGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCCTGACG	1860
30	CATCCCGGCT	ATGCAGCCCC	AGTCCAGGGC	AGCAAGGCAG	GCCCCGCTG	CCTCTTCACC	1920
	CGGAGGCCTC	TGCCCGCCCC	ACTCATGTCT	AGGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG	1980
	GCTCTGGGCA	GGCACAGGCT	AGGTGCCCCCT	AACCCAGGCC	CTGCACACAA	AGGGGCAGGT	2040
	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC	CGGGAGGACC	CTGCCCTGA	CCTAAGCCCA	2100
	CCCCAAAGGC	CAAACTCTCC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA	2160
35	GTAATCCCA	ATCTTCTCTC	TGCAGAGCCC	AAATCTTGTG	ACAAAACCTCA	CACATGCCCA	2220
	CCGTGCCCA	GTAAGCCAGC	CCAGGCTCTG	CCCTCCAGCT	CAAGGCGGGA	CAGGTGCCCT	2280
	AGATAGCCT	GCATCCAGGG	ACAGGCCCCA	GCCGGGTGCT	GACACGTCCA	CCTCCATCTC	2340
	TTCTCAGCA	CCTGAACTCC	TGGGGGGACC	GTCAGTCTTC	CTCTTCCCCC	CAAAACCCAA	2400
	GGACACCCTC	ATGATCTCCC	GGACCCCTGA	GGTCACATGC	GTGGTGGTGG	ACGTGAGCCA	2460
40	CGAAGACCT	GAGGTCAAGT	TCAACTGGTA	CGTGGACGGC	GTGGAGGTGC	ATAATGCCAA	2520
	GACAAAGCCG	CGGGAGGAGC	AGTACAACAG	CACGTACCGT	GTGGTCAGCG	TCCTCACCGT	2580
	CCTGCACCA	GACTGGCTGA	ATGGCAAGGA	GTACAAGTGC	AAGGTCTCCA	ACAAAGCCCT	2640
	CCCAGCCCCC	ATCGAGAAAA	CCATCTCCAA	AGCCAAAGGT	GGGACCCGTG	GGGTGCGAGG	2700
	GCCACATGGA	CAGAGGCCGG	CTCGGCCAC	CCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	2760
45	CCTCTGTCCC	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA	CACCTGCCCC	CCATCCCGGG	2820
	ATGAGCTGAC	CAAGAACCCAG	GTCAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCAGCG	2880
	ACATCGCCGT	GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG	ACCACGCCTC	2940
	CCGTGCTGGA	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA	GCTCACCGTG	GACAAGAGCA	3000
	GGTGGCAGCA	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	3060
50	ACACGCAGAA	GAGCCTCTCC	CTGTCTCCGG	GTAAATGAGT	GCGACGGCCG	GCAAGCCCCC	3120
	GCTCCCCGGG	CTCTCGCGGT	CGCACGAGGA	TGCTTGGCAC	GTACCCCTTG	TACATACTTC	3180
	CCGGGCGCCC	AGCATGGAAA	TAAAGCACCC	AGCGCTGCCC	TGGGCCCCCTG	CGAGACTGTG	3240
	ATGGTTCTTT	CCACGGGTCA	GGCCGAGTCT	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	3300
	GGGTCCCACT	GTCCCCACAC	TGGCCGAGGC	TGTGCAAGTG	TGCCCTGGGC	CCCTAGGGTG	3360
55	GGGCTCAGCC	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	3420
	AGCAGCACCT	GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCTTGGG	GACAGACACA	3480
	CAGCCCCCTG	CTCTGTAGGA	GACTGTCTTG	TTCTGTGAGC	GCCCTGTGTC	TCCCGACCTC	3540
	CATGCCCCACT	CGGGGGCATG	CCTAGTCCAT	GTGCGTAGGG	ACAGGCCCTC	CCTCACCCAT	3600
	CTACCCCCAC	GGCACTAACC	CCTGGCTGCC	CTGCCAGCC	TCCGACCCGC	ATGGGGACAC	3660

	AACCGACTCC	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GA CTGGTGCA	GATGCCCACA	3720
	CACACACTCA	GCCCAGACCC	GTTCAACAAA	CCCCGCACTG	AGGTTGGCCG	GCCACACGGC	3780
	CACCACACAC	ACACGTGCAC	GCCTCACACA	CGGAGCCTCA	CCCGGGCGAA	CTGCACAGCA	3840
	CCCGAGCCAG	AGCAAGGTCC	TCGCACACGT	GAACACTCCT	CGGACACAGG	CCCCACGAG	3900
5	CCCCACGCGG	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	3960
	TCAGACAAAC	CCAGCCCTCC	TCTCACAAGG	GTGCCCCCTG	AGCCGCCACA	CACACACAGG	4020
	GGATCACACA	CCACGTACAG	TCCCTGGCCC	TGGCCCACTT	CCCAGTGCCG	CCCTTCCCTG	4080
	CAGGACGGAT	CAGCCTCGAC	TGTGCCTTCT	AGTTGCCAGC	CATCTGTTGT	TTGCCCCCTC	4140
	CCCGTGCCCT	CCTTGACCCT	GGAAGGTGCC	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	4200
10	GAAATTGCAT	CGCATTGTCT	GAGTAGGTGT	CATTCTATTG	TGGGGGGTGG	GGTGGGGCAG	4260
	GACAGCAAGG	GGGAGGATTG	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	4320
	ATGGCTTCTG	AGGCGGAAAG	AACCAGCTGG	GGCTCTAGGG	GGTATCCCCA	CGCGCCCTGT	4380
	AGCGGCGCAT	TAAGCGCGGC	GGGTGTGGTG	GTTACGCGCA	GCGTGACCGC	TACACTTGCC	4440
	AGCGCCCTAG	CGCCCGCTCC	TTTCGCTTTC	TTCCCTTCTT	TTCTCGCCAC	GTTCCGCGGG	4500
15	CCTCTCAAAA	AAGGGA AAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	4560
	CTAACTCCGC	CCATCCC GCC	CCTAACTCCG	CCCAGTTCGG	CCCATTCTCC	GCCCCATGGC	4620
	TGACTAATTT	TTTTTATTTA	TGCAGAGGCC	GAGGCCGCTT	CGGCCCTCTG	GCTATTCCAG	4680
	AAGTAGTGAG	GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	4740
	GCTGCGATTT	CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	4800
20	CCCGCTGCCA	TCATGGTTCG	ACCATTTGAA	TGCATCGTCG	CCGTGTCCCA	AAATATGGGG	4860
	ATTGGCAAGA	ACGAGACCTT	ACCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	4920
	AGAATGACCA	CAACCTCTTC	AGTGGAAGGT	AAACAGAATC	TGGTGATTAT	GGGTAGGAAA	4980
	ACCTGGTTCT	CCATTCTCTG	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	5040
	AGTAGAGAAC	TCAAAGAACC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG	TTTGGATGAT	5100
25	GCCTTAAGAC	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	5160
	GGAGGCAGTT	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	5220
	ACAAGGATCA	TGCAGGAATT	TGAAAGTGAC	ACGTTTTTCC	CAGAAATTGA	TTTGGGGAAA	5280
	TATAAACTTC	TCCCAGAATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	5340
	AAGTATAAGT	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	5400
30	GCTCCCCTCC	TAAAGCTATG	CATTTTTATA	AGACCATGGG	ACTTTTGCTG	GCTTTAGATC	5460
	TCTTTGTGAA	GGAACCTTAC	TTCTGTGGTG	TGACATAATT	GGACAACTA	CCTACAGAGA	5520
	TTTAAAGCTC	TAAGGTAAAT	ATAAAATTTT	TAAGTGATATA	ATGTGTTAAA	CTACTGATTC	5580
	TAATTGTTTG	TGTATTTTAG	ATTCCAACCT	ATGGAACCTG	TGAATGGGAG	CAGTGGTGGA	5640
	ATGCCTTTAA	TGAGGAAAAC	CTGTTTGTCT	CAGAAGAAAT	GCCATCTAGT	GATGATGAGG	5700
35	CTACTGCTGA	CTCTCAACAT	TCTACTCCTC	CAAAAAGAA	GAGAAAGGTA	GAAGACCCCA	5760
	AGGACTTTCC	TTCAGAATTG	CTAAGTTTTT	TGAGTCATGC	TGTGTTTAGT	AATAGAACTC	5820
	TTGCTTGCTT	TGCTATTTAC	ACCACAAAGG	AAAAGCTGCG	ACTGCTATAC	AAGAAAATTA	5880
	TGGAAAATAA	TTCTGTAACC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	5940
	TTTTTCTTAC	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACTATGCT	CAAAAATTGT	6000
40	GTACCTTTAG	CTTTTAAATT	TGTAAAGGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCCT	6060
	TGACTAGAGA	TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTACTTTGC	TTTAAAAAAC	6120
	CTCCACACCC	TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	6180
	TTTATTGCAG	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	6240
	GCATTTTTTT	CACGTGATTC	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	6300
45	GTCTGGATCG	GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCCAC	6360
	CCCAACTTGT	TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTT	6420
	ACAAATAAAG	CATTTTTTTC	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	6480
	TCTTATCATG	TCTGTATACC	GTGACCTCTT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	6540
	CTGTTTCTTG	TGTGAAATTG	TTATCCGCTC	ACAATTCACG	ACAACATACG	AGCCGGAAGC	6600
50	ATAAAGTGTA	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	6660
	TCACTGCCCG	CTTTCAGTGC	GGGAAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	6720
	CGCGCGGGGA	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCGG	CTTCCTCGCT	CACTGACTCG	6780
	CTGCGCTCGG	TCGTTCCGGT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	6840
	TTATCCACAG	AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	6900
55	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	6960
	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	7020
	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	7080
	ACCGGATACC	TGTCGCGCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTCTCTA	ATGCTCACGC	7140
	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	7200

	CCCGTTCAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	7260
	AGACACGACT	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	7320
	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	7380
	GTATTTGGTA	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	7440
5	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	7500
	ACGCGCAGAA	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	7560
	CAGTGAACG	AAAACTCACG	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	7620
	ACCTAGATCC	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	7680
	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	7740
10	TTTCGTTTCA	CCATAGTTGC	CTGACTCCCC	GTCGTGTAGA	TAACTACGAT	ACGGGAGGGC	7800
	TTACCATCTG	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	7860
	TTATCAGCAA	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	7920
	TCCGCCTCCA	TCCAGTCTAT	TAATTGTGTG	CGGGAAGCTA	GAGTAAGTAG	TTCCGCCAGTT	7980
	AATAGTTTGC	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACG	CTCGTCGTTT	8040
15	GGTATGGCTT	CATTGAGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	8100
	TTGTGCAAAA	AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTGAGAAG	TAAGTTGGCC	8160
	GCAGTGTTAT	CACTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	8220
	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	8280
	CGGCGACCGA	GTTGCTCTTG	CCCGCGCTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	8340
20	ACTTTAAAG	TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC	GAAAACTCTC	AAGGATCTTA	8400
	CCGCTGTTGA	GATCCAGTTC	GATGTAAACC	ACTCGTGCAC	CCAAGTATC	TTCAGCATCT	8460
	TTTACTTTCA	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAAG	8520
	GGAATAAGGG	CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTTCA	ATATTATTGA	8580
	AGCATTTATC	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	8640
25	AAACAAATAG	GGGTTCCGCG	CACATTTCCT	CGAAAAGTGC	CACCTGACGT	C	8691

## (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8327 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

	GACGGATCGG	GAGATCTGCT	AGGTGACCTG	AGGCGCGCCG	GCTTCGAATA	GCCAGAGTAA	60
40	CCTTTTTTTT	TAATTTTATT	TTATTTTATT	TTTGAGATGG	AGTTTGGCGC	CGATCTCCCG	120
	ATCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGCGC	GAGCAAAATT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAACTCTGCT	TAGGGTTAGG	CGTTTTGCGC	300
	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAAT	360
45	AGTAATCAAT	TACGGGGTCA	TTAGTTCATA	GCCCATATAT	GGAGTTCGCG	GTTACATAAC	420
	TTACGGTAAA	TGGCCGCGCT	GGCTGACCGC	CCACGACCC	CCGCCCATG	ACGTCAATAA	480
	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTTACGGTA	AACTGCCCAC	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCC	CCTGGCATTG	TGCCCAGTAC	ATGACCTTAT	660
50	GGGACTTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	720
	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	AATGTCGTAA	CAACTCGGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	960
55	TTAATACGAC	TCACTATAGG	GAGACCCAAG	CTTGGTACCA	ATTTAAATTG	ATATCTCCTT	1020
	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGGCGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCCT	TGTTTTAAAA	GGTGCCAGT	1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
	TCTCCTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATTA	CATGTATTGG	GTTCCGCCAGA	1260

	CTCCAGAGAA	GAGGCTGGAG	TGGGTCGCAT	ACATTAGTCA	AGGTGGTGAT	ATAACCGACT	1320
	ATCCAGACAC	TGTAAAGGGT	CGATTACCA	TCTCCAGAGA	CAATGCCAAG	AACACCCTGT	1380
	ACCTGCAAAAT	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500
5	CTAGACACAA	GGCCCCATCG	GTCTTCCCC	TGGCACCTC	CTCCAAGAGC	ACCTCTGGGG	1560
	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCGGTG	ACGGTGTCTG	1620
	GGAACTCAGG	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCTTA	CAGTCCTCAG	1680
	GACTCTACTC	CCTCAGCAGC	GTGGTCACCG	TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCT	1740
	ACATCTGCAA	CGTGAATCAC	AAGCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTGGGTGAGA	1800
10	GGCCAGCACA	GGGAGGGAGG	GTGTCTGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCTGGACG	1860
	CATGCCCGCT	ATGCAGCCCC	AGTCCAGGGC	AGCAAGGCAG	GCCCCGTCTG	CCTCTTCACC	1920
	CGGAGGCTC	TGCCCCGCCC	ACTCATGCTC	AGGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG	1980
	GCTCTGGGCA	GGCACAGGCT	AGGTGCCCT	AACCCAGGCC	CTGCACACAA	AGGGGCAGGT	2040
	GCTGGGTCA	GACCTGCCAA	GAGCCATATC	CGGGAGGACC	CTGCCCTGA	CCTAAGCCCA	2100
15	CCCCAAAGGC	CAAACCTCTC	ACTCCCTCAG	CTCGGACACC	TTCTCTCTCT	CCAGATTCCA	2160
	GTAACCTCCA	ATCTTCTCTC	TGCAGAGCCC	AAATCTTGTG	ACAAACTCA	CACATGCCCA	2220
	CCGTGCCGAG	GTAAAGCCAGC	CCAGGCCCTG	CCCTCCAGCT	CAAGGCGGGA	CAGGTGCCCT	2280
	AGAGTAGCCT	GCATCCAGGG	ACACACCACG	TGGGTACCAA	CATGTCCGGA	GCCACATGGA	2340
	CAGAGGCCGG	CTGGGCCAC	CCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	CCTCTGTCCC	2400
20	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA	CACCTGCCCC	CCATCCCGGG	ATGAGCTGAC	2460
	CAAGAACCAG	GTGAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCACGCG	ACATCGCCGT	2520
	GGAGTGGGAG	AGCAATGGGC	AGCGGAGAA	CAACTACAAG	ACCACGCCTC	CGGTGCTGGA	2580
	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA	GCTCACCGTG	GACAAGAGCA	GGTGGCAGCA	2640
	GGGGAACGTC	TTCTCATGCT	CGGTGATGCA	TGAGGCTCTG	CACAACCACT	ACACGCAGAA	2700
25	GAGCCTCTCC	CTGTCTCCGG	GTAAATGAGT	GCGACGGCCG	GCAAGCCCCC	GCTCCCCGGG	2760
	CTCTCGCGGT	CGCACGAGGA	TGCTTGGCAC	GTACCCCTCG	TACATACTTC	CCGGGCGCCC	2820
	AGCATGGAAA	TAAAGCACCC	AGCGCTGCCC	TGGGCCCTCG	CGAGACTGTG	ATGGTTCTTT	2880
	CCACGGGTCA	GGCCGAGTCT	GAGGCCGTGAG	TGGCATGAGG	GAGGCAGAGC	GGGTCCCACT	2940
	GTCCCCACAC	TGGCCACGGC	TGTGCAGGTG	TGCCTGGGCC	CCCTAGGGTG	GGGCTCAGCC	3000
30	AGGGGCTGCC	CTGGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	AGCAGCACCT	3060
	GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCCCTGG	GACAGACACA	CAGCCCCCTG	3120
	CTCTGTAGGA	GACTGTCTCT	TTCTGTGAGC	GCCCCGTGTC	TCCCGACCTC	CATGCCCACT	3180
	CGGGGGCATG	CTTAGTCCAT	GTGCGTAGGG	ACAGGCCCTC	CCTCACCCAT	CTACCCCCAC	3240
	GGCACTAACC	CCTGGCTGCC	CTGCCACGCC	TCGCACCCGC	ATGGGGACAC	AACCGACTCC	3300
35	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GACTGGTGCA	GATGCCACAC	CACACACTCA	3360
	GCCCAGACCC	GTTCACAAA	CCCCGCACTG	AGGTGGCCCG	GCCACACGGC	CACCACACAC	3420
	ACACGTGCAC	GCCTCACACA	CGGAGCCTCA	CCCCGGCGAA	CTGCACAGCA	CCCAGACCAG	3480
	AGCAAGGTCC	TGCACACGT	GAACACTCCT	CGGACACAGG	CCCCCAGGAG	CCCCACGGG	3540
	CACCTCAAGG	CCACAGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	TCAGACAAAC	3600
40	CCAGCCCTCC	TCTCACAAAG	GTGCCCTGCG	AGCCGCCACA	CACACACAGG	GGATCACACA	3660
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	CAGCCTCGAC	TGTGCCTTCT	AGTTGCCAGC	CATCTGTTGT	TTGCCCTCC	CCCGTGCCCT	3780
	CCTTGACCCT	GGAAGGTGCC	ACTCCCCTG	TCCTTTCTTA	ATAAAATGAG	GAAATTGCAT	3840
	CGCATTGTCT	GAGTAGGTGT	CATTCTATTC	TGGGGGGTGG	GGTGGGGCAG	GACAGCAAGG	3900
45	GGGAGGATTG	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	ATGGCTTCTG	3960
	AGGCGGAAAG	AACCAGCTGG	GGCTCTAGGG	GGTATCCCCA	CGCGCCCTGT	AGCGGCGCAT	4020
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	CGCCCGCTCC	TTTCGCTTTC	TTCCCTTCT	TTCTCGCCAC	GTTCGCGGGG	CCTCTCAAAA	4140
	AAGGGAAAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	CTAACTCCGC	4200
50	CCATCCCGCC	CCTAACTCCG	CCCAGTTCGG	CCCATTCTCC	GCCCCATGGC	TGACTAATTT	4260
	TTTTTATTTA	TGCAGAGGCC	GAGGCCGCCT	CGGCCTCTGA	GCTATTCCAG	AAGTAGTGAG	4320
	GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	GCTGCGATTT	4380
	CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	CCCGCTGCCA	4440
	TCATGGTTCT	ACCATTGAAC	TGCATCGTGC	CCGTGTCCCA	AAATATGGGG	ATTGGCAAGA	4500
55	ACGGAGACCT	ACCCTGGCCT	CGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	AGAATGACCA	4560
	CAACCTCTTC	AGTGGAAAGT	AAACAGAAATC	TGGTGATTAT	GGGTAGGAAA	ACCTGGTTCT	4620
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	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	GGAGGCAGTT	4800

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5	TAAAGCTATG	CATTTTATA	AGACCATGGG	ACTTTTGCTG	GCTTTAGATC	TCTTTGTGAA	5100
	GGAACCTTAC	TTCTGTGGTG	TGACATAATT	GGACAACTA	CCTACAGAGA	TTTAAAGCTC	5160
	TAAGGTAAAT	ATAAAATTTT	TAAGTGATATA	ATGTGTTAAA	CTACTGATTC	TAATTGTTTG	5220
	TGTATTTTAG	ATTCCAACCT	ATGGAACCTGA	TGAATGGGAG	CAGTGGTGGA	ATGCCTTTAA	5280
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10	CTCTCAACAT	TCTACTCCTC	CAAAAAAGAA	GAGAAAGGTA	GAAGACCCCA	AGGACTTTCC	5400
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	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAATATGCT	CAAAAATTGT	GTACCTTTAG	5640
15	CTTTTTAATT	TGTAAAGGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCCT	TGACTAGAGA	5700
	TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTTACTTGC	TTTAAAAAAC	CTCCACACC	5760
	TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	TTTATTGCAG	5820
	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATT	CACAAATAAA	GCATTTTTTT	5880
	CACTGCATTC	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	GTCTGGATCG	5940
20	GCTGGATGAT	CCTCCAGCGC	GGGATCTCA	TGCTGGAGTT	CTTCGCCCAC	CCCAACTTGT	6000
	TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTC	ACAAATAAAG	6060
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	TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTTCCTG	6180
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25	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	TCCTGCCCCG	6300
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	GAGGCGGTTT	GCGTATTGGG	CGCTCTCCG	CTTCTCGCT	CACTGACTCG	CTGCGCTCGG	6420
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35	CCGACCGCTG	CGCCTTATCC	GGTAACATATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	6900
	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	6960
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## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 8897 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## 10 (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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   TCATTGTACA TAATAATGGC AACACCTATT TAGAATGGTA CCTGCAGAAA CCAGGCCAGT      240
   CTCCACAGCT CCTGATCTAC AAAGTTTCCA ACCGATTTTC TGGGGTCCCA GACAGGTTCA      300
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	CTCATCATTC	GAAAACGTTT	TTCCGGGCGA	AACTCTCAA	GGATCTTACC	GCTGTTGAGA	7620
	TCCAGTTCGA	TGTAACCCAC	TCTGTCACCC	AACTGATCTT	CAGCATCTTT	TACTTTTACC	7680
	AGCGTTTCTG	GGTGAGCAAA	AACAGGAAGG	CAAAATGCCG	CAAAAAAGGG	AATAAGGGCG	7740
25	ACACGGAAAT	GTTGAATACT	CATACTCTTC	CTTTTTCAAT	ATTATTGAAG	CATTTATCAG	7800
	GGTTATTGTC	TCATGAGCGG	ATACATATTT	GAATGTATTT	AGAAAAATAA	ACAAATAGGG	7860
	GTTCCGCGCA	CATTTCCCCG	AAAAGTGCCA	CCTGACGTCG	ACGGATCGGG	AGATCTGCTA	7920
	GCCCGGGTGA	CCTGAGGCGC	GCCGGCTTCG	AATAGCCAGA	GTAACCTTTT	TTTTTAATTT	7980
	TATTTTATTT	TATTTTGTAG	ATGGAGTTTG	GCGCCGATCT	CCCGATCCCC	TATGGTCGAC	8040
30	TCTCAGTACA	ATCTGCTCTG	ATGCCGCATA	GTTAAGCCAG	TATCTGCTCC	CTGCTTGTGT	8100
	GTTGGAGGTC	GCTGAGTAGT	GCGCGAGCAA	AATTTAAGCT	ACAACAAGGC	AAGGCTTGAC	8160
	CGACAATTGC	ATGAAGAATC	TGCTTAGGGT	TAGGCGTTTT	GCGCTGCTTC	GCGATGTACG	8220
	GGCCAGATAT	ACGCGTTGAC	ATTGATTATT	GACTAGTTAT	TAATAGTAAT	CAATTACGGG	8280
	GTCATTAGTT	CATAGCCCAT	ATATGGAGTT	CCGCGTTACA	TAACTTACGG	TAAATGGCCC	8340
35	GCCTGGCTGA	CCGCCCAACG	ACCCCCGCCC	ATTGACGTCA	ATAATGACGT	ATGTTCCCAT	8400
	AGTAACGCCA	ATAGGGACTT	TCCATTGACG	TCAATGGGTG	GACTATTTAC	GGTAAACTGC	8460
	CCACTTGGCA	GTACATCAAG	TGTATCATAT	GCCAAGTACG	CCCCCTATTG	ACGTCAATGA	8520
	CGGTAAATGG	CCCGCCTGGC	ATTATGCCCA	GTACATGACC	TTATGGGACT	TTCTTACTTG	8580
	GCAGTACATC	TACGTATTAG	TCATCGCTAT	TACCATGGTG	ATGCGGTTTT	GCGAGTACAT	8640
40	CAATGGGCGT	GGATAGCGGT	TTGACTCACG	GGGATTTCCA	AGTCTCCACC	CCATTGACGT	8700
	CAATGGGAGT	TTGTTTTGGC	ACCAAAATCA	ACGGGACTTT	CCAAAATGTC	GTAACAACCTC	8760
	CGCCCCATTG	ACGCAAATGG	GCGGTAGGCG	TGTACGGTGG	GAGGTCTATA	TAAGCAGAGC	8820
	TCTCTGGCTA	ACTAGAGAAC	CCACTGCTTA	CTGGCTTATC	GAAATTAATA	CGACTCACTA	8880
45	TAGGGAGACC	CAAGCTT					8897

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8321 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC GGTCAATCGA 60  
 TTGGAATTCT TGCGGCCGCT TGCTAGCCAC CATGGAGTTG TGGTTAAGCT TGGTCTTCCT 120

	TGTCCTTGTT	TAAAAAGGTG	TCCAGTGTGA	AGTGCAACTG	GTGGAGTCTG	GGGGAGGCTT	180
	AGTGCGAGCT	GGAGGGTCCC	TGCGACTTTC	CTGTGCTGCA	TCTGGATTCC	CGTTCAGTGA	240
	CTATTACATG	TATTGGGTTC	GCCAGGCTCC	AGGCAAGGGA	CTGGAGTGGG	TCTCATACAT	300
	TAGTCAAGAT	GGTGATATAA	CCGACTATGC	AGACTCCGTA	AAGGGTCGAT	TCACCATCTC	360
5	CAGAGACAAT	GCAAGAACA	GCCTGTACCT	GCAATGAAC	AGCCTGAGGG	ACGAGGACAC	420
	AGCCGTGTAT	TACTGTGCAA	GAGGCCTGGC	GGACGGGGCC	TGGTTTGCTT	ACTGGGGCCA	480
	AGGGACTCTG	GTACCGGTCT	CTTCCGCTAG	CACCAAGGGC	CCATCGGTCT	TCCCCCTGGC	540
	ACCCTCCTCC	AAGAGCACCT	CTGGGGGCAC	AGCGGCCCTG	GGCTGCCTGG	TCAAGGACTA	600
	CTTCCCCGAA	CCGGTGACGG	TGTCGTGAA	CTCAGGCGCC	CTGACCAGCG	GCGTGCACAC	660
10	CTTCCCGGCT	GTCCTACAGT	CCTCAGGACT	CTACTCCCTC	AGCAGCGTGG	TCACCGTGCC	720
	CTCCAGCAGC	TTGGGCACCC	AGACCTACAT	CTGCAACGTG	AATCACAAGC	CCAGCAACAC	780
	CAAGGTGGAC	AAGAAAGTTG	GTGAGAGGCC	AGCACAGGGA	GGGAGGGTGT	CTGCTGGAAG	840
	CCAGGCTCAG	CGCTCCTGCC	TGGACGCATC	CCGGCTATGC	AGCCCCAGTC	CAGGGCAGCA	900
	AGGCAAGCCCC	CGTCTGCCTC	TTCAACCCGA	GGCCTCTGCC	CGCCCCACTC	ATGCTCAGGG	960
15	AGAGGGTCTT	CTGGCTTTTT	CCCCAGGCTC	TGGGCAGGCA	CAGGCTAGGT	GCCCCTAACC	1020
	CAGGCCCTGC	ACACAAAGGG	GCAGGTGCTG	GGCTCAGACC	TGCCAAGAGC	CATATCCGGG	1080
	AGGACCCCTG	CCCTGACCTA	AGCCACCCCC	AAAGGCCAAA	CTCTCCACTC	CCTCAGCTCG	1140
	GACACCTTCT	CTCCTCCAG	ATTCCAGTAA	CTCCCAATCT	TCTCTCTGCA	GAGCCCAAT	1200
	CTTGTGACAA	AACTCACACA	TGCCCCACGT	GCCCAGGTAA	GCCAGCCCAG	GCCTCGCCCT	1260
20	CCAGCTCAAG	GCGGGACAGG	TGCCCTAGAG	TAGCCTGCAT	CCAGGGACAC	ACCAGCTGGG	1320
	TACCAACATG	TCCGGAGCCA	CATGGACAGA	GGCCGGCTCG	GCCCCACCCTC	TGCCCTGAGA	1380
	GTGACCGCTG	TACCAACCTC	TGTCCCTACA	GGGCAGCCCC	GAGAACCACA	GGTGTACACC	1440
	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG	AACCAGGTCA	GCCTGACCTG	CCTGGTCAAA	1500
	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	1560
25	TACAAGACCA	CGCCTCCCGT	GCTGGACTCC	GACGGCTCCT	TCTTCTCTA	CAGCAAGCTC	1620
	ACCGTGGACA	AGAGCAGGTG	GCAGCAGGGG	AACGTCTTCT	CATGCTCCGT	GATGCATGAG	1680
	GCTCTGCACA	ACCACTACAC	GCAGAAGAGC	CTCTCCCTGT	CTCCGGGTAA	ATGAGTGCGA	1740
	CGGCCGGCAA	GCCCCCGCTC	CCCGGGCTCT	CGCGGTGCGA	CGAGGATGCT	TGGCACGTAC	1800
	CCCCTGTACA	TACTTCCCGG	GCGCCAGCA	TGGAATAAA	GCACCCAGCG	CTGCCCTGGG	1860
30	CCCCTGCGAG	ACTGTGATGG	TTCTTTCCAC	GGGTGAGGCC	GAGTCTGAGG	CCTGAGTGGC	1920
	ATGAGGGAGG	CAGAGCGGGT	CCCCTGTCC	CCCACTGGC	CCAGGCTGTG	CAGGTGTGCC	1980
	TGGGCCCCCT	AGGGTGGGGC	TCAGCCAGGG	GCTGCCCTCG	GCAGGGTGGG	GGATTGTCCA	2040
	GCGTGGCCCT	CCCTCCAGCA	GCACCTGCCC	TGGGCTGGGC	CACGGGAAGC	CCTAGGAGCC	2100
	CCTGGGGACA	GACACACAGC	CCCTGCCTCT	GTAGGAGACT	GTCCTGTTCT	GTGAGCGCCC	2160
35	CTGTCTCTCC	GACCTCCATG	CCCACTCGGG	GGCATGCCTA	GTCCATGTGC	GTAGGGACAG	2220
	GCCCTCCCTC	ACCCATCTAC	CCCCACGGCA	CTAACCCCTG	GCTGCCCTGC	CCAGCCTCGC	2280
	ACCCGCTATG	GGACACAACC	GACTCCGGGG	ACATGCACTC	TCCGGCCCTG	TGGAGGGACT	2340
	GGTGCAGATG	CCCACACACA	CACTCAGCCC	AGACCCGTTT	AACAAACCCC	GCACTGAGGT	2400
	TGGCCGGCCA	CACGGCCACC	ACACACACAC	GTGCACGCCT	CACACACGGA	GCCTCACCCG	2460
40	GGCGAACTGC	ACAGCACCCA	GACCAGAGCA	AGGTCTCTGC	ACACGTGAAC	ACTCCTCGGA	2520
	CACAGGCCCC	CACGAGCCCC	ACGCGGCACC	TCAAGGCCCA	CGAGCCTCTC	GGCAGCTTCT	2580
	CCACATGCTG	ACCTGCTCAG	ACAAACCCAG	CCCTCCTCTC	ACAAGGGTGC	CCCTGCAGCC	2640
	GCCACACACA	CACAGGGGAT	CACACACCAC	GTCACGTCCC	TGGCCCTGGC	CCACTTCCCA	2700
	GTGCCGCCCT	TCCCTGCAGG	ACGGATCAGC	CTCGACTGTG	CCTTCTAGTT	GCCAGCCATC	2760
45	TGTTGTTTGC	CCCTCCCCCG	TGCCTTCCTT	GACCCTGGAA	GGTGCCACTC	CCACTGTCTT	2820
	TTCCTAATAA	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCATT	CTATTCTGGG	2880
	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA	GACAATAGCA	GGCATGCTGG	2940
	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAACC	AGCTGGGGCT	CTAGGGGGTA	3000
	TCCCCACGCG	CCCTGTAGCG	GCGCATTAAG	CGCGGCGGGT	GTGGTGGTTA	CGCGCAGCGT	3060
50	GACCGCTACA	CTTGCCAGCG	CCCTAGCGCC	CGCTCCTTTC	GCTTCTCTCC	CTTCCTTTCT	3120
	CGCCACGTTT	GCCGGGCCCTC	TCAAAAAAGG	GAAAAAAGC	ATGCATCTCA	ATTAGTCAGC	3180
	AACCATAGTC	CCGCCCTTAA	CTCCGCCCAT	CCCGCCCTTA	ACTCCGCCCA	GTTCCGCCCA	3240
	TTCTCCGCCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA	GAGGCCGAGG	CCGCCTCGGC	3300
	CTCTGAGCTA	TTCCAGAAAT	AGTGAGGAGG	CTTTTTTGGA	GGCCTAGGCT	TTTGCAAAAA	3360
55	GCTTGGACAG	CTCAGGGCTG	CGATTTCGCG	CCAACTTGA	CGGCAATCCT	AGCGTGAAGG	3420
	CTGGTAGGAT	TTTATCCCCG	CTGCCATCAT	GGTTCGACCA	TTGAACTGCA	TCGTGCGCGT	3480
	GTCCCAAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC	TCAGGAACGA	3540
	GTTCAAGTAC	TTCCAAAGAA	TGACCACAAC	CTCTTCAGTG	GAAGGTAAAC	AGAATCTGGT	3600
	GATTATGGGT	AGGAAAACCT	GGTTCTCCAT	TCCTGAGAAG	AATCGACCTT	TAAAGGACAG	3660

	AATTAATATA	GTTCTCAGTA	GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	ATTTTCTTGC	3720
	CAAAAGTTTG	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	GTAAAGTAGA	3780
	CATGGTTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAGGAA	GCCATGAATC	AACCAGGCCA	3840
	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAATTTGAA	AGTGACACGT	TTTTCCAGAG	3900
5	AATTGATTTG	GGGAAATATA	AACTTCTCCC	AGAATACCCA	GGCGTCTCT	CTGAGGTCCA	3960
	GGAGGAAAAA	GGCATCAAGT	ATAAGTTTGA	AGTCTACGAG	AAGAAAGACT	AACAGGAAGA	4020
	TGCTTTCAAG	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	CATGGGACTT	4080
	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC	ATAATTGGAC	4140
	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAAATATAA	AATTTTTAAG	TGTATAATGT	4200
10	GTAAACTAC	TGATTCTAAT	TGTTTGTGTA	TTTTAGATTG	CAACCTATGG	AACTGATGAA	4260
	TGGGAGCAGT	GGTGAATGCG	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	AGAAATGCCA	4320
	TCTAGTGATG	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	AAAGAAGAGA	4380
	AAGGTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCTAA	GTTTTTTGAG	TCATGCTGTG	4440
	TTTAGTAATA	GAACCTTTCG	TTGCTTTGCT	ATTACACCA	CAAAGGAAAA	AGCTGCACCTG	4500
15	CTATACAAGA	AAATTATGGA	AAAATATTCT	GTAACTTTTA	TAAGTAGGCA	TAACAGTTAT	4560
	AATCATAACA	TACTGTTTTT	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	TATTAATAAC	4620
	TATGCTCAAA	AATTGTGTAC	CTTTAGCTTT	TTAATTTGTA	AAGGGGTTAA	TAAGGAATAT	4680
	TTGATGTATA	GTGCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTGG	TAGAGGTTTTT	4740
	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA	TGAATGCAAT	4800
20	TGTTGTGTGT	AACCTGTTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAGCA	ATAGCATCAC	4860
	AAATTTTACA	AATAAAGCAT	TTTTTTTCACT	GCATTCTAGT	TGTGGTTTGT	CCAAACTCAT	4920
	CAATGTATCT	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	ATCTCATGCT	4980
	GGAGTTCTTC	GCCCAACCCA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA	AATAAAGCAA	5040
	TAGCATCACA	AATTTTCAAA	ATAAAGCATT	TTTTTCACTG	CATTCTAGTT	GTGGTTTGTG	5100
25	CAAACTCATC	AATGTATCTT	ATCATGTCTG	TATACCGTCG	ACCTCTAGCT	AGAGCTTGCC	5160
	GTAATCATGG	TCATAGCTGT	TTCTGTGTG	AAATTGTTAT	CCGCTCACA	TTCCACACAA	5220
	CATACGAGCC	GGAAAGCATA	AGTGTAAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAACTCAC	5280
	ATTAATTGCG	TTGCGCTCAC	TGCCCGCTTT	CCAGTCGGGA	AACCTGTCTG	GCCAGCTGCA	5340
	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCGT	ATTGGGCGCT	CTTCGCTTTC	5400
30	CTCGCTCACT	GACTCGCTGC	GCTCGGTCGT	TCCGCTGCGG	CGAGCGGTAT	CAGCTCACTC	5460
	AAAGGCGGTA	ATACGGTTAT	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	5520
	AAAAGGCCAG	CAAAGGCCCA	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	TTTTCCATAG	5580
	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCCGACGCTA	AGTCAGAGGT	GGCGAAACCC	5640
	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC	TCCCTCGTGC	GCTCTCCTGT	5700
35	TCCGACCCTG	CCGCTTACCG	GATACCTGTC	CGCCTTTCTC	CCTTCGGGAA	GCGTGGCGCT	5760
	TTCTCAATGC	TCACGCTGTA	GGTATCTCAG	TTCCGCTGTAG	GTGCTTCGCT	CCAAGCTGGG	5820
	CTGTGTGCAC	GAACCCCCCG	TTACGCCCCG	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT	5880
	TGAGTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG	GTAAACAGGT	5940
40	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC	CTAACTACGG	6000
	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	6060
	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAACA	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	6120
	TTGCAAGCAG	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	6180
	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	TCATGAGATT	6240
	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATTAAAAA	TGAAGTTTAA	AATCAATCTA	6300
45	AAGTATATAT	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	6360
	CTCAGCGATC	TGTCTATTTT	GTTTATCCAT	AGTTGCCTGA	CTCCCGTCTG	TGTAGATAAC	6420
	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGTGCA	ATGATACCGC	GAGACCCACG	6480
	CTCACC GGCT	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGGAGGGCCG	AGCGCAGAAG	6540
	TGGTCCTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	AAGCTAGAGT	6600
50	AAGTAGTTTG	CCAGTTAATA	GTTTGCAGAA	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT	6660
	GTCACGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	6720
	TACATGATCC	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC	TTCGGTCCCT	CGATCGTTGT	6780
	CAGAAAGTAA	TTGGCCGCAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC	ATAATTCTCT	6840
	TACTGTTCATG	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	6900
55	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG	CTCTTGCCCG	GCGTCAATAC	GGGATAATAC	6960
	CGCGCCACAT	AGCAGAACTT	TAAAAAGTCT	CATCATTTGA	AAACGTTCTT	CGGGGCGAAA	7020
	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTCTGATG	TAACCCACTC	GTGCACCCAA	7080
	CTGATCTTCA	GCATCTTTTA	CTTTCACCAG	CGTTTCTGGG	TGAGCAAAAA	CAGGAAGGCA	7140
	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	TACTCTTCCT	7200

	TTTTCAATAT	TATTGAAGCA	TTTATCAGGG	TTATTGTCTC	ATGAGCGGAT	ACATATTTGA	7260
	ATGTATTTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	AAGTGCCACC	7320
	TGACGTGCAC	GGATCGGGAG	ATCTGCTAGG	TGACCTGAGG	CGCGCCGGCT	TCGAATAGCC	7380
	AGAGTAACCT	TTTTTTTTAA	TTTTATTTTA	TTTTATTTT	GAGATGGAGT	TTGGCGCCGA	7440
5	TCTCCCGATC	CCCTATGGTC	GACTCTCAGT	ACAATCTGCT	CTGATGCCGC	ATAGTTAAGC	7500
	CAGTATCTGC	TCCCTGCTTG	TGTGTTGGAG	GTCGCTGAGT	AGTGCGCGAG	CAAAATTTAA	7560
	GCTACAACAA	GGCAAGGCTT	GACCGACAAT	TGCATGAAGA	ATCTGCTTAG	GGTTAGGCGT	7620
	TTTGCGCTGC	TTCCGCGATGT	ACGGGCCAGA	TATACGCGTT	GACATTGATT	ATTGACTAGT	7680
	TATTAATAGT	AATCAATTAC	GGGGTCATTA	GTTTCATAGCC	CATATATGGA	GTTCCGCGTT	7740
10	ACATAACTTA	CGGTAAATGG	CCCGCCTGGC	TGACCGCCCA	ACGACCCCGG	CCCATTGACG	7800
	TCAATAATGA	CGTATGTTCC	CATAGTAACG	CCAATAGGGA	CTTTCATTG	ACGTCAATGG	7860
	GTGGACTATT	TACGGTAAAC	TGCCCCACTT	GCAGTACATC	AAGTGATCA	TATGCCAAGT	7920
	ACGCCCCCTA	TTGACGTCAA	TGACGGTAAA	TGGCCCCGCT	GGCATTATGC	CCAGTACATG	7980
	ACCTTATGGG	ACTTTCCTAC	TTGGCAGTAC	ATCTACGTAT	TAGTCATCGC	TATTACCATG	8040
15	GTGATGCGGT	TTTGGCAGTA	CATCAATGGG	CGTGGATAGC	GGTTTGACTC	ACGGGGATTT	8100
	CCAAGTCTCC	ACCCCATTTGA	CGTCAATGGG	AGTTTGTTTT	GGCACCAAAA	TCAACGGGAC	8160
	TTTCCAAAT	GTCGTAACAA	CTCCGCCCCA	TTGACGCAAA	TGGGCGGTAG	GCGTGTACGG	8220
	TGGGAGGTCT	ATATAAGCAG	AGCTCTCTGG	CTAACTAGAG	AACCCACTGC	TTACTGGCTT	8280
20	ATCGAAATTA	ATACGACTCA	CTATAGGGAG	ACCCAAGCTT	G		8321

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8897 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

	GACGGATCGG	GAGATCTGCT	AGCCCGGGTG	ACCTGAGGCG	CGCCGGCTTC	GAATAGCCAG	60
	AGTAACCTTT	TTTTTTAATT	TTATTTTATT	TTATTTTGA	GATGGAGTTT	GGCGCCGATC	120
35	TCCCGATCCC	CTATGGTCGA	CTCTCAGTAC	AATCTGCTCT	GATGCCGCAT	AGTTAAGCCA	180
	GTATCTGCTC	CCTGCTTGTC	TGTTGGAGGT	CGCTGAGTAG	TGCGCGAGCA	AAATTTAAGC	240
	TACAACAAGG	CAAGGCTTGA	CCGACAATTG	CATGAAGAAT	CTGCTTAGGG	TTAGGCGTTT	300
	TGCGCTGCTT	CGCGATGTAC	GGGCCAGATA	TACGCGTTGA	CATTGATTAT	TGACTAGTTA	360
	TTAATAGTAA	TCAATTACGG	GGTCATTAGT	TCATAGCCCA	TATATGGAGT	TCCGCGTTAC	420
40	ATAACTTACG	GTAATGGGCC	CGCCTGGCTG	ACCGCCCAAC	GACCCCGGCC	CATTGACGTC	480
	AATAATGACG	TATGTTCCCA	TAGTAACGCC	AATAGGGACT	TTCCATTGAC	GTCAATGGGT	540
	GGACTATTTA	CGGTAAACTG	CCCACTTGCC	AGTACATCAA	GTGTATCATA	TGCCAAGTAC	600
	GCCCCCTATT	GACGTCAATG	ACGGTAAATG	GCCCGCCTGG	CATTATGCCC	AGTACATGAC	660
	CTTATGGGAC	TTTCCTACTT	GGCAGTACAT	CTACGTATTA	GTCATCGCTA	TTACCATGGT	720
45	GATGCGGTTT	TGGCAGTACA	TCAATGGGCG	TGGATAGCGG	TTTGAATCAC	GGGGATTTCC	780
	AAGTCTCCAC	CCCATTGACG	TCAATGGGAG	TTTGTTTTGG	CACCAAAATC	AACGGGACTT	840
	TCCAAAATGT	CGTAACAAC	CCGCCCCATT	GACGCAAATG	GGCGGTAGGC	GTGTACGGTG	900
	GGAGGTCTAT	ATAAGCAGAG	CTCTCTGGCT	AACTAGAGAA	CCCACTGCTT	ACTGGCTTAT	960
	CGAAATTAAT	ACGACTCACT	ATAGGGAGAC	CCAAGCTTGG	TACCAATTTA	AATTGATATC	1020
50	TCCTTAGGTC	TCGAGCACCA	TGAAGTTGCC	TGTTAGGCTG	TTGGTGCTGA	TGTTCTGGAT	1080
	TCCTGCTTCC	AGCAGTGATG	TTGTCTATGAC	CCAAACCCCA	CTGTCCAGTC	CTGTACGCT	1140
	TGGACAACCT	GCGTCCATCT	CTTGCAATC	TAGTCAGATC	ATTGTACATA	ATAATGGCAA	1200
	CACCTATCTG	GAATGGTACC	AGCAGAGACC	AGGCAGTCT	CCACGGCTCC	TGATCTACAA	1260
	AGTTTCCAAC	CGATTTTCTG	GGGTCCCAGA	CAGGTTGAGC	GGCAGTGGAG	CTGGGACAGA	1320
55	TTTCACACTC	AAGATCAGCA	GAGTGGAGGC	TGAGGATGTG	GGAGTTTACT	ACTGCTTCCA	1380
	GGGTTACAT	GTTCCATTCA	CGTTCGGCCA	AGGGACAAAG	TTGGAAATCA	AACGTAAGTC	1440
	TCGAGTCTCT	AGATAACCGG	TCAATCGATT	GGAATTCTAA	ACTCTGAGGG	GGTCGGATGA	1500
	CGTGGCCATT	CTTTGCCTAA	AGCATTGAGT	TTACTGCAAG	GTCAGAAAAG	CATGCAAAGC	1560
	CCTCAGAATG	GCTGCAAAGA	GCTCCAACAA	AACAATTTAG	AACTTTATTA	AGGAATAGGG	1620

	GGAGCTAGG	AAGAACTCA	AAACATCAAG	ATTTTAAATA	CGCTTCTTGG	TCTCCTTGCT	1680
	ATAATTATCT	GGGATAAGCA	TGCTGTTTTT	TGTCGTGCCC	TAACATGCCC	TTATCCGCAA	1740
	ACAACACACC	CAAGGGCAGA	ACTTTGTTC	TTAAACACCA	TCCTGTTTGC	TTCTTTCCTC	1800
5	AGGAACTGTG	GCTGCACCAT	CTGTCTTCAT	CTTCCCAGCA	TCTGATGAGC	AGTTGAAATC	1860
	TGGAAGTGGC	TCTGTTGTGT	GCCTGCTGAA	TAACCTCTAT	CCCAGAGAGG	CCAAAGTACA	1920
	GTGGAAGGTG	GATAACGCCC	TCCAATCGGG	TAACCTCCAG	GAGAGTGTCA	CAGAGCAGGA	1980
	GAGCAAGGAC	AGCACCTACA	GCCTCAGCAG	CACCCTGACG	CTGAGCAAAG	CAGACTACGA	2040
	GAAACACAAA	GTCTACGCCT	GCGAAGTCAC	CCATCAGGGC	CTGAGCTCGC	CCGTACACAA	2100
	GAGCTTCAAC	AGGGGAGAGT	GTTAGAGGGA	GAAGTGCCCC	CACCTGCTCC	TCAGTTCAG	2160
10	CCTGACCCCC	TCCCATCCTT	TGGCCTCTGA	CCCTTTTTC	ACAGGGGACC	TACCCCTATT	2220
	GCGTCCCTCC	AGCTCATCTT	TCACCTCACC	CCCCCTCCTC	TCCTTGGCTT	TAATTATGCT	2280
	AATGTTGGAG	GAGAATGAAT	AAATAAAGTG	AATCTTTGCA	CCTGTGGTTT	CTCTCTTTCC	2340
	TCATTTAATA	ATTATTATCT	GTTGTTTTAC	CACTACTCA	ATTTCTCTTA	TAAGGGACTA	2400
	AATATGTAGT	CATCCTAAGG	CACGTAACCA	TTTATAAAAA	TCATCCTTCA	TTCTATTTTA	2460
15	CCCTATCATC	CTCTGCAAGA	CAGTCTCTCC	TCAAACCCAC	AAGCCTTCTG	TCCTCACAGT	2520
	CCCCTGGGCC	ATGGTAGGAG	AGACTTGCTT	CCTGTGTTTT	CCCTCCTCAG	CAAGCCCTCA	2580
	TAGTCTTTTT	TAAGGGTGAC	AGGTCTTACA	GTCATATATC	CTTTGATTCA	ATTCCCTGAG	2640
	AATCAACCAA	AGCAAATTTT	TCAAAGAAG	AAACCTGCTA	TAAAGAGAAT	CATTCAATTG	2700
	AACATGATAT	AAAATAACAA	CACAATAAAA	GCAATTAAAT	AAACAAACAA	TAGGGAAATG	2760
20	TTTAAGTTCA	TCATGGTACT	TAGACTTAAT	GGAATGTCAT	GCCTTATTTA	CATTTTAA	2820
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	TAATCCACAC	TATACGTGTA	GATTAAAAAC	ATTCTATAAA	ATGTTGCAAA	GGTCTATAA	2940
	AGCTGAGAGA	CAATATATTT	CTATAACTCA	GCAATCCAC	TTCTAGATGA	CTGAGTGCTC	3000
	CCACCCACCA	AAAACTATG	CAAGAATGTT	CAAGCAGCT	TTATTACAA	AAGCCAAAAA	3060
25	TTGGAATAG	CCCGATTGTC	CAACAATAGA	ATGAGTTATT	AAACTGTGGT	ATGTTTATAC	3120
	ATTTAGAATAC	CCAATGAGGA	GAATTAACAA	GCTACAATA	TACCTACTCA	CACAGATGAA	3180
	TCTCATAAAA	ATAATGTTAC	ATAAGAGAAA	CTCAATGCAA	AAGATATGTT	CTGTATGTTT	3240
	TCATCCATAT	AAAGTTCAAA	ACCAGGTAAA	AATAAAGTTA	GAAATTTGGA	TGGAAATTAC	3300
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30	TTGGTAATGT	TCTGTTCTCT	GTGTGGGGTT	GTGCAGTTAT	GATCTGTGCA	CTGTTCTGTA	3420
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	GAAGAGGAAT	AAGTAATAGG	TCAAGACCAA	CGCAGCTGGT	AAGTGGGGGC	CTGGGATCAA	3540
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	GGGAAGGGCA	CATGTAAATG	AGGACTCTTC	CTCATCTAT	GGGGCACTCT	GGCCCTGCC	3840
	CTCTCAGCTA	CTCATCCATC	CAACACACCT	TTCTAAGTAC	CTCTCTCTGC	CTACACTCTG	3900
	AAGGGGTTCA	GGAGTAACTA	ACACAGCATC	CCTTCCCTCA	AATGACTGAC	AATCCCTTTG	3960
40	TCCTGCTTTG	TTTTTCTTTT	CAGTCAGTAC	TGGGAAAGTG	GGGAAGGACA	GTCTAGGAGA	4020
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AAGTGCCACC	TGACGTC					8897



What is claimed is:

1. A method for inhibiting immunoglobulin-induced toxicity resulting from  
5 immunoglobulin immunotherapy in a subject comprising administering an  
immunoglobulin molecule to the subject, the immunoglobulin molecule  
having a variable region and a constant region, the immunoglobulin molecule  
being modified prior to administration by structurally altering multiple  
toxicity associated domains in the constant region so that immunoglobulin-  
10 induced toxicity is inhibited.
2. A method for inhibiting immunoglobulin-induced toxicity resulting from  
immunoglobulin immunotherapy in a subject comprising administering a  
structurally altered antibody to the subject, the structurally altered antibody  
15 comprising a variable region and a constant region, multiple toxicity  
associated domains in the constant region being modified so as to render the  
constant region unable to mediate an ADCC response or activate  
complement thereby inhibiting immunoglobulin-induced toxicity resulting  
from immunotherapy.
- 20 3. A method for inhibiting immunoglobulin-induced toxicity resulting from  
immunotherapy in a subject comprising administering an Ig fusion protein to  
the subject, the Ig fusion protein having multiple structurally altered toxicity  
associated domains in the constant region.
- 25 4. A method for inhibiting immunoglobulin-induced toxicity resulting from  
immunotherapy in a subject comprising administering an Ig fusion protein to  
the subject, the Ig fusion protein comprising a modified constant region, the

modification being a structural alteration in multiple toxicity associated regions within the CH<sub>2</sub> domain.

5. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
- (a) selecting an immunoglobulin which recognizes and binds a target, the target being associated with the disease;
  - (b) mutating the immunoglobulin so selected by structurally altering multiple toxicity associated domains in the constant region of the immunoglobulin thereby creating a structurally altered immunoglobulin;
  - (c) administering the structurally altered immunoglobulin of step (b) to the subject under conditions so that the structurally altered immunoglobulin recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the constant region thereby preventing immunoglobulin-induced toxicity in the subject.
6. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
- (a) selecting an Ig fusion protein which recognizes and binds a target, the target being associated with the disease;
  - (b) structurally altering multiple toxicity associated domains in the CH<sub>2</sub> domain of the constant region of the Ig protein so selected;

- 5 (c) administering the structurally altered Ig fusion protein of step (b) to the subject under conditions so that the structurally altered Ig fusion protein recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the CH<sub>2</sub> domain thereby preventing immunoglobulin-induced toxicity in the subject.
- 10 7. The method of claim 1, 2, 3, 4, 5, or 6, wherein the portion of the constant region is the CH<sub>2</sub> domain.
8. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgG.
9. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgM.
- 15 10. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgA.
11. The method of claim 2, wherein the antibody recognizes and binds Le<sup>y</sup>.
- 20 12. The method of claim 2, wherein the antibody recognizes and binds to Le<sup>x</sup>.
13. The method of claim 2, wherein the antibody is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
- 25 14. The method of claim 2, wherein the antibody is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.

15. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds Le<sup>y</sup>.
16. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds to Le<sup>x</sup>.
17. The method of claim 1 or 5, wherein the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
18. The method of claim 1 or 5, wherein the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
19. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds Le<sup>y</sup>.
20. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds to Le<sup>x</sup>.
21. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
22. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
23. A pharmaceutical composition comprising a pharmaceutically effective

amount of a structurally altered immunoglobulin, and an acceptable carrier, the structurally altered immunoglobulin (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH<sub>2</sub> domain.

- 5    24.    A pharmaceutical composition comprising a pharmaceutically effective amount of structurally altered Ig fusion protein, and an acceptable carrier, the structurally altered Ig fusion protein (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH<sub>2</sub> domain.
- 10   25.    A method of treating carcinomas in vivo comprising administering to a subject a pharmaceutically effective amount of the composition of claim 23 or 24.
- 15   26.    The method of claim 30, wherein the structurally altered immunoglobulin in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.
- 20   27.    The method of claim 24, wherein the Ig fusion protein in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.
- 25   28.    The method of claim 2 or 5, wherein the antibody is conjugated to a cytotoxic agent.
29.    The method of claim 1, wherein the immunoglobulin is conjugated to a cytotoxic agent.

30. The method of claim 3, 4 or 6, wherein the Ig fusion protein is conjugated to a cytotoxic agent.
31. The method of claim 28, 29, or 31, wherein the cytotoxic agent is selected  
5 from the group consisting of antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents.
32. A method for treating a subject suffering from a cancer, the cancer being  
10 characterized as a group of cells having a tumor associated antigen on the cell surface, which method comprises administering to the subject a cancer killing amount of the composition of claim 23 or 24 joined to a cytotoxic agent under conditions which permit the molecule so joined to bind the tumor associated antigen on the cell surface so as to kill the cells so bound  
15 thereby curing the subject.
33. A pharmaceutical composition comprising a pharmaceutically effective amount of a structurally altered BR96 antibody, the structurally altered antibody having an inactivated CH<sub>2</sub> domain.  
20
34. A method for treating a subject suffering from a proliferative type disease characterized by cells having a BR96 antigen on the cell surface which comprises administering to the subject an effective amount of the composition of claim 33 joined to doxorubicin such that the  
25 immunoconjugate binds the BR96 antigen and kills said cells thereby treating the subject.
35. A method for inhibiting BR96 (ATCC: HB10036) induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering

BR96 to the subject, the BR96 molecule being modified prior to administration, the modification comprising the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated toxicity is inhibited.

36. A method for preventing BR96 (ATCC: HB10036) induced toxicity resulting from immunotherapy for cancer in a subject comprising:
- (a) mutating the BR96 polypeptide by the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated immunoglobulin-induced toxicity is inhibited in the altered BR96 polypeptide; and
  - (b) administering the structurally altered BR96 polypeptide of step (a) to the subject under conditions so that the peptide recognizes and binds cancer associated Le<sup>y</sup> antigens, thereby alleviating symptoms associated with the cancer, the structural alteration of the toxicity associated domains thereby preventing BR96 toxicity in the subject.
37. A chimeric BR96 antibody having a structurally altered constant region having the CH1 and CH3 domains but not the CH2 domain, the antibody being designated cBR96-A.

38. The chimeric BR96 antibody of claim 37 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 10.
39. A BR96 antibody having humanized variable and constant regions, wherein the constant region has been structurally altered so that the CH1 and CH3 domains are present but the CH2 domain is not, the antibody being designated hBR96-2A.
40. The BR96 antibody of claim 39 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 12.
41. A BR96 antibody designated hBR96-2B having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine and glycine at amino acid position 237 is mutated to alanine.
42. A BR96 antibody designated hBR96-2C having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.
43. A BR96 antibody designated hBR96-2D having a structurally altered constant region wherein proline at amino acid position 331 is mutated to alanine.
44. A BR96 antibody designated hBR96-2E having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid



position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.

45. A BR96 antibody designated hBR96-2F having a structurally altered  
5 constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; and proline at amino acid position 331 is mutated to alanine.
46. A BR96 antibody designated hBR96-2G having a structurally altered  
10 constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
47. A BR96 antibody designated hBR96-2H having a structurally altered  
15 constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; lysine at amino acid position 322 is  
20 mutated to serine; and proline at amino acid position 331 is mutated to alanine.
48. A nucleic acid molecule which encodes the BR96 antibody of claim 37, 39,  
25 and 41-47.
49. A cDNA of claim 48.
50. A plasmid which comprises the nucleic acid molecule of claim 48.

51. A host vector system comprising a plasmid of claim 50 in a suitable host cell.
52. A method for producing a protein comprising growing the host vector system of claim 51 so as to produce the protein in the host and recovering the protein
- 5 so produced.

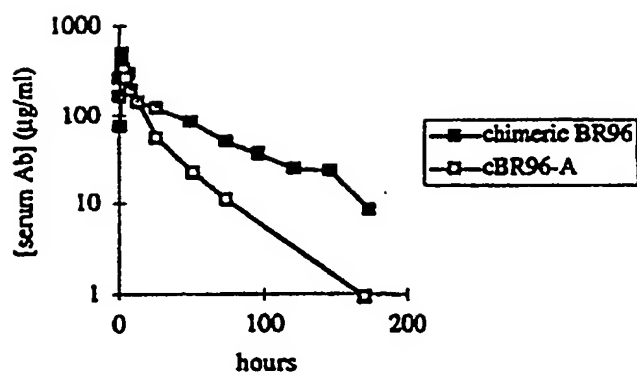


Figure 1. Plasma clearance in high LeY expressing dogs chimeric versus constant region mutant of cBR96-2.

Figure One

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Figure 2

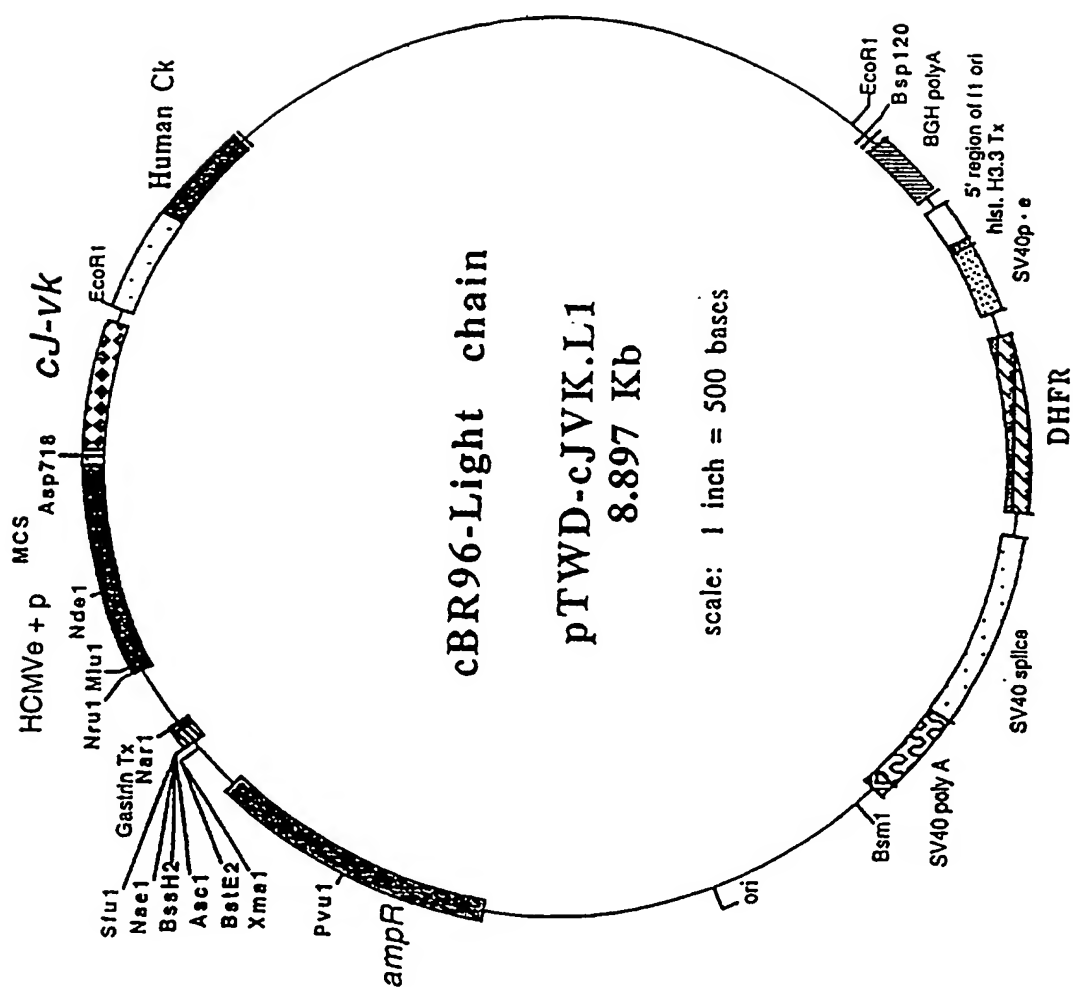


Figure 3

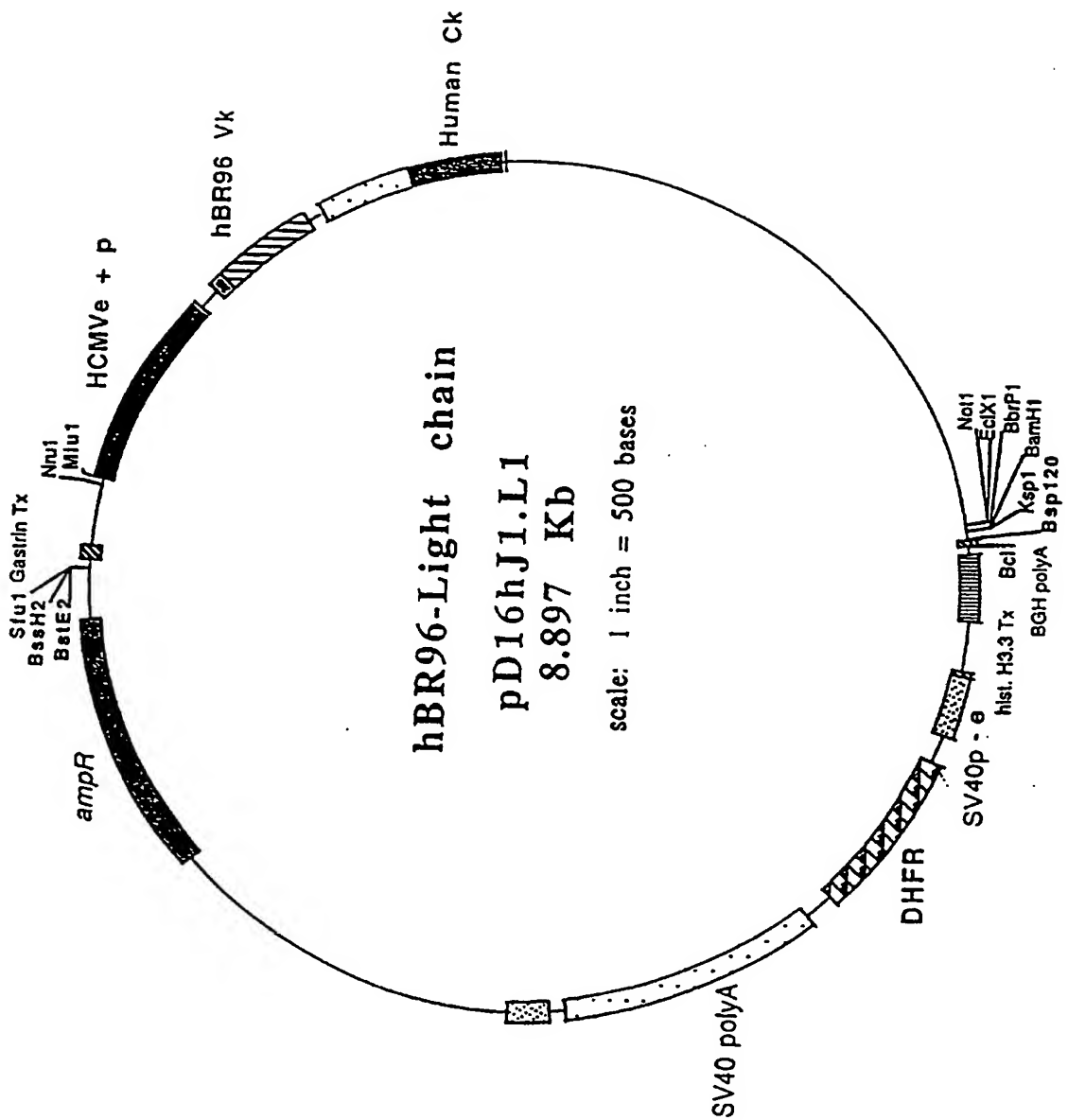


Figure 4

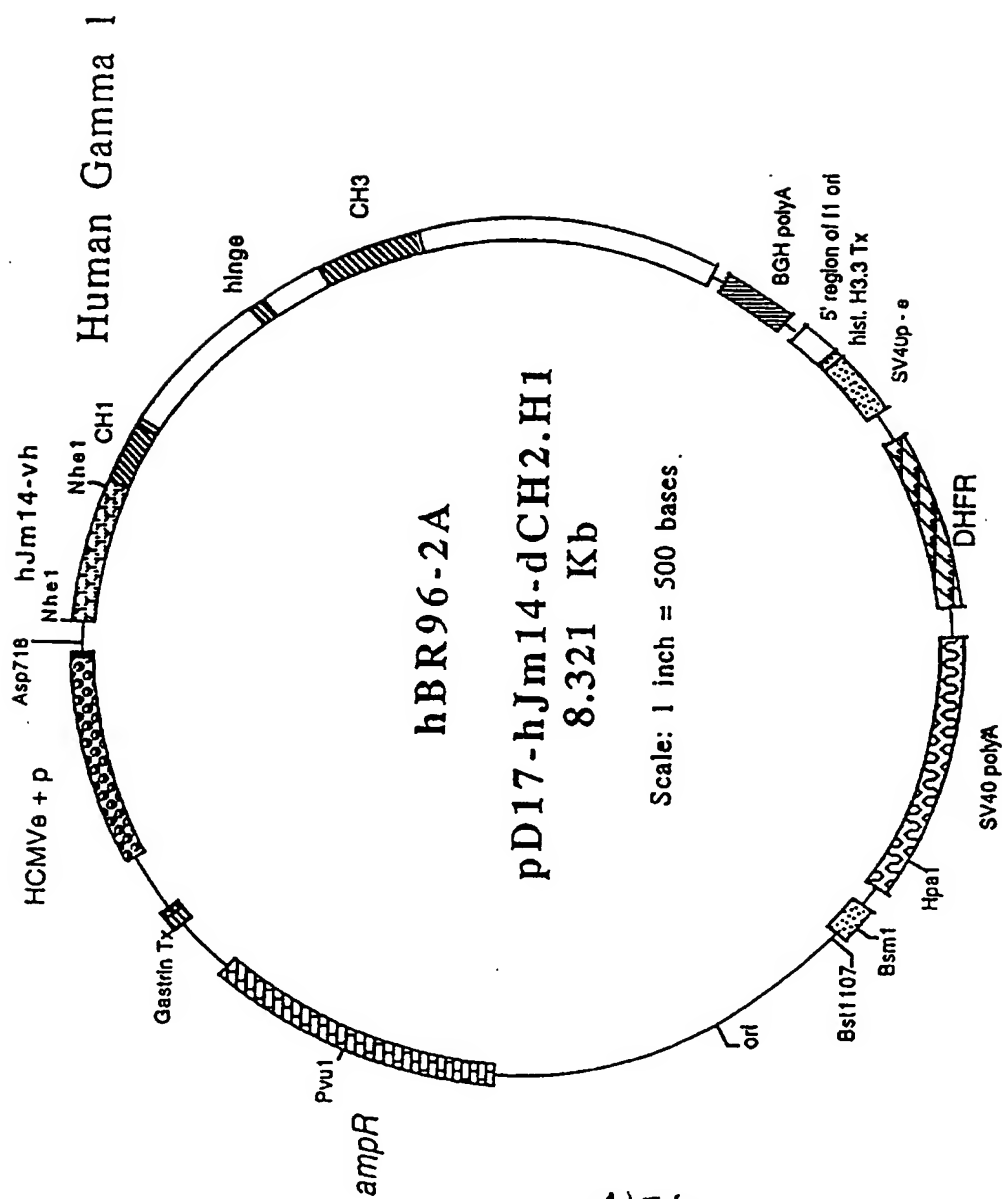


Figure 5

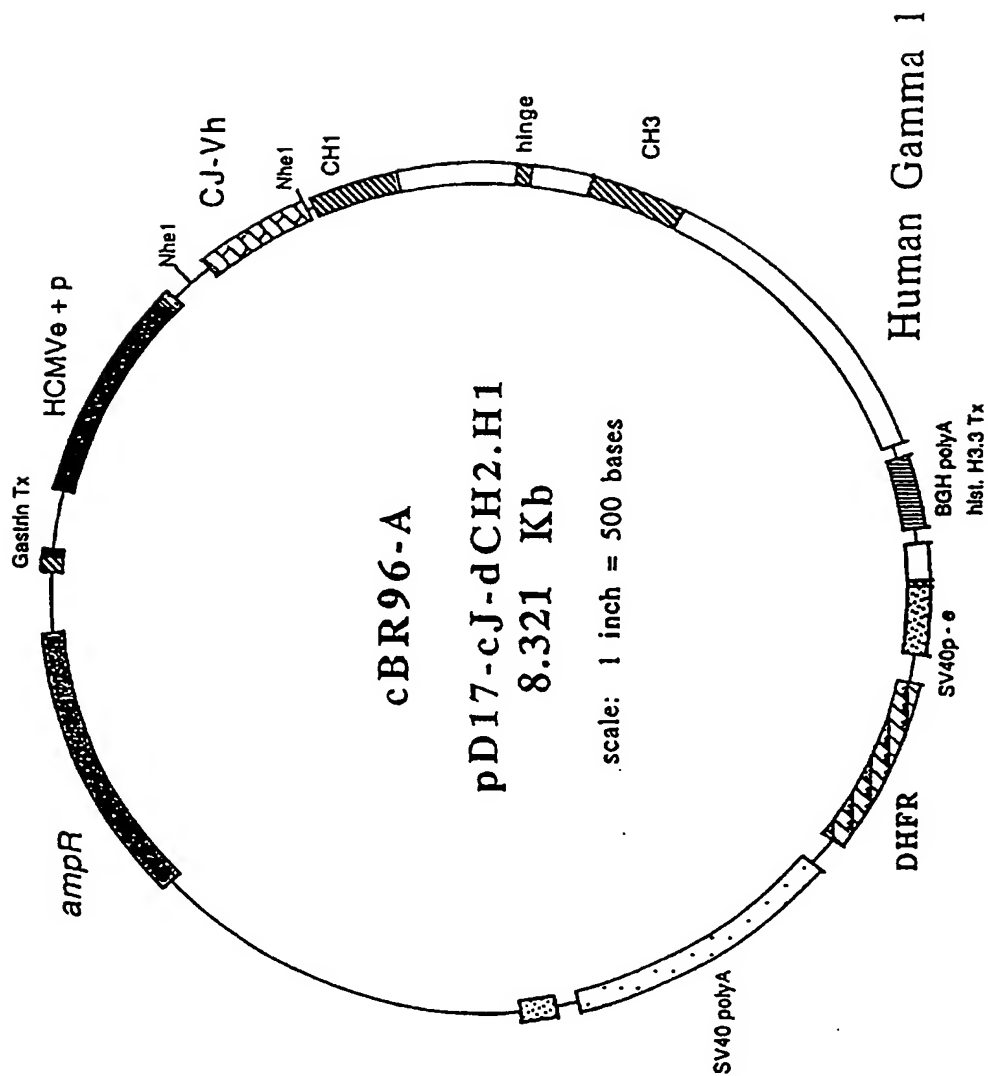
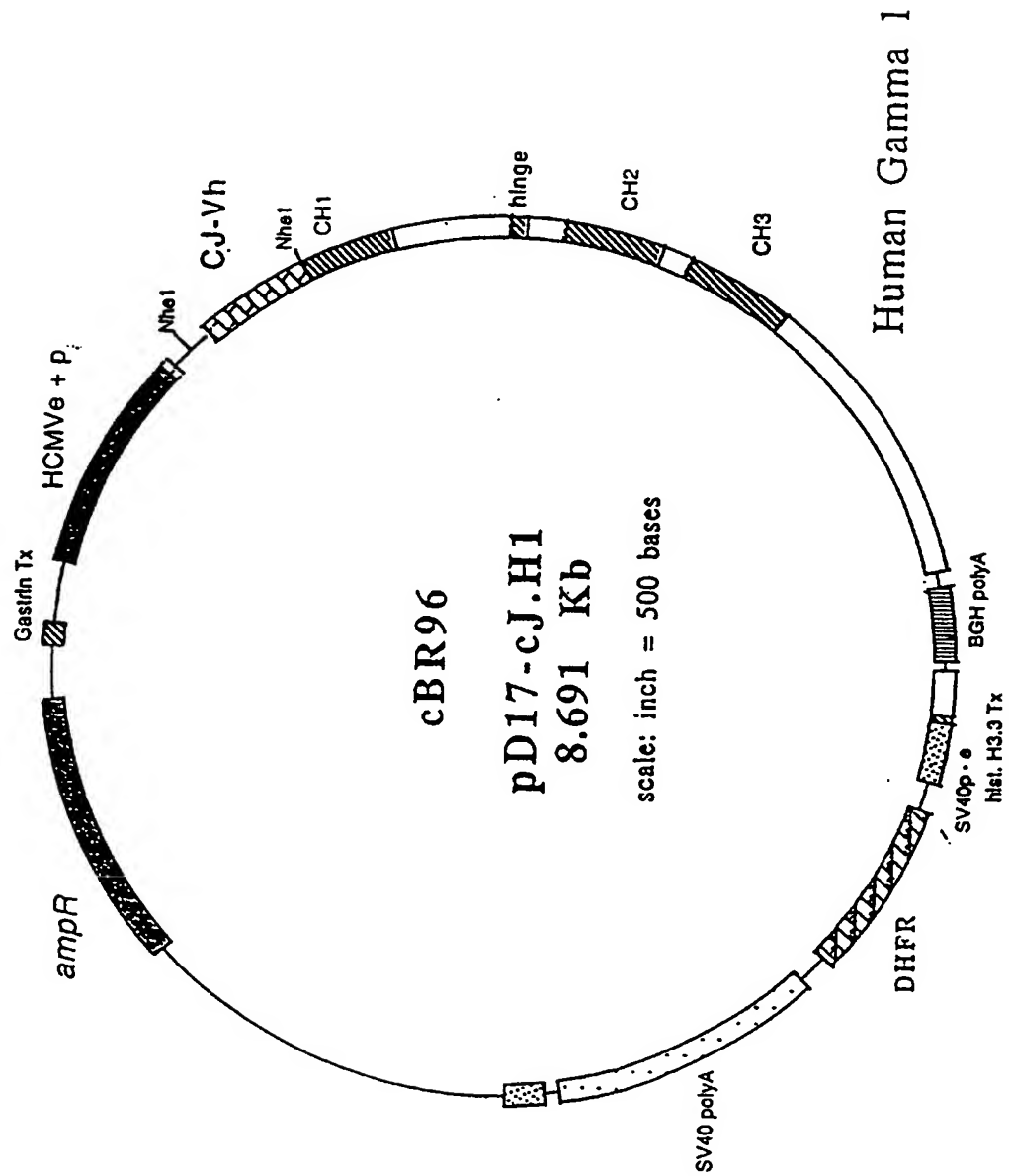


Figure 6



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Figure 7

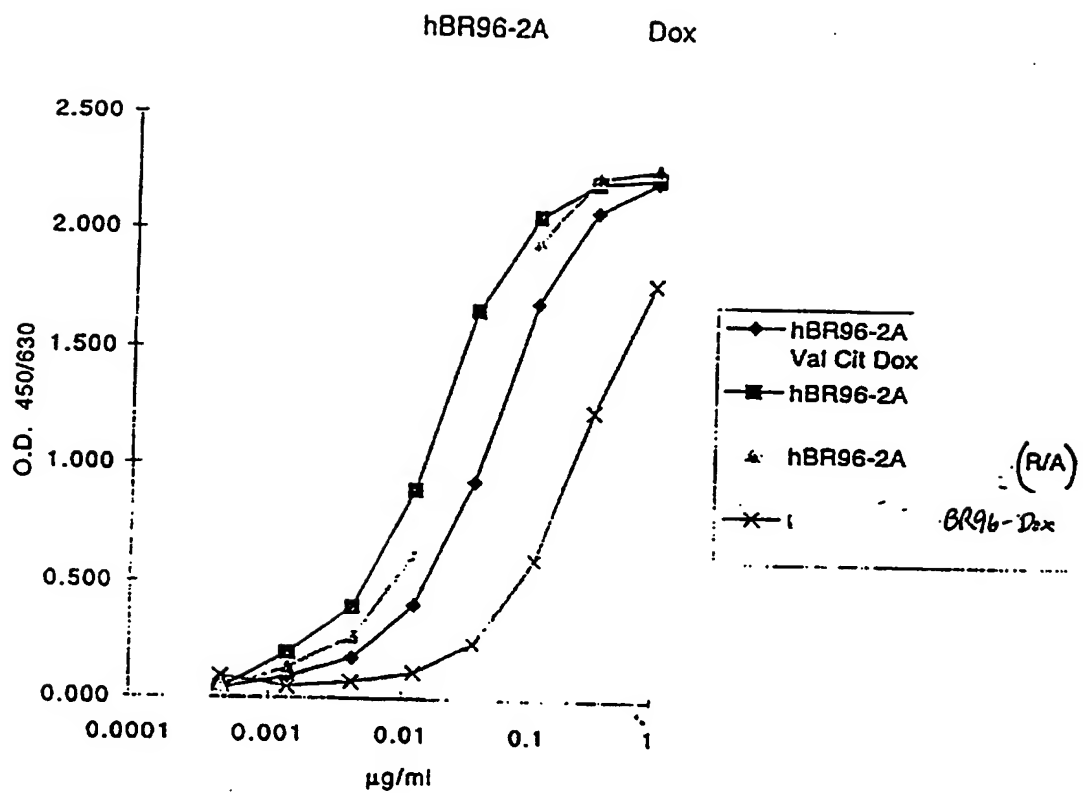
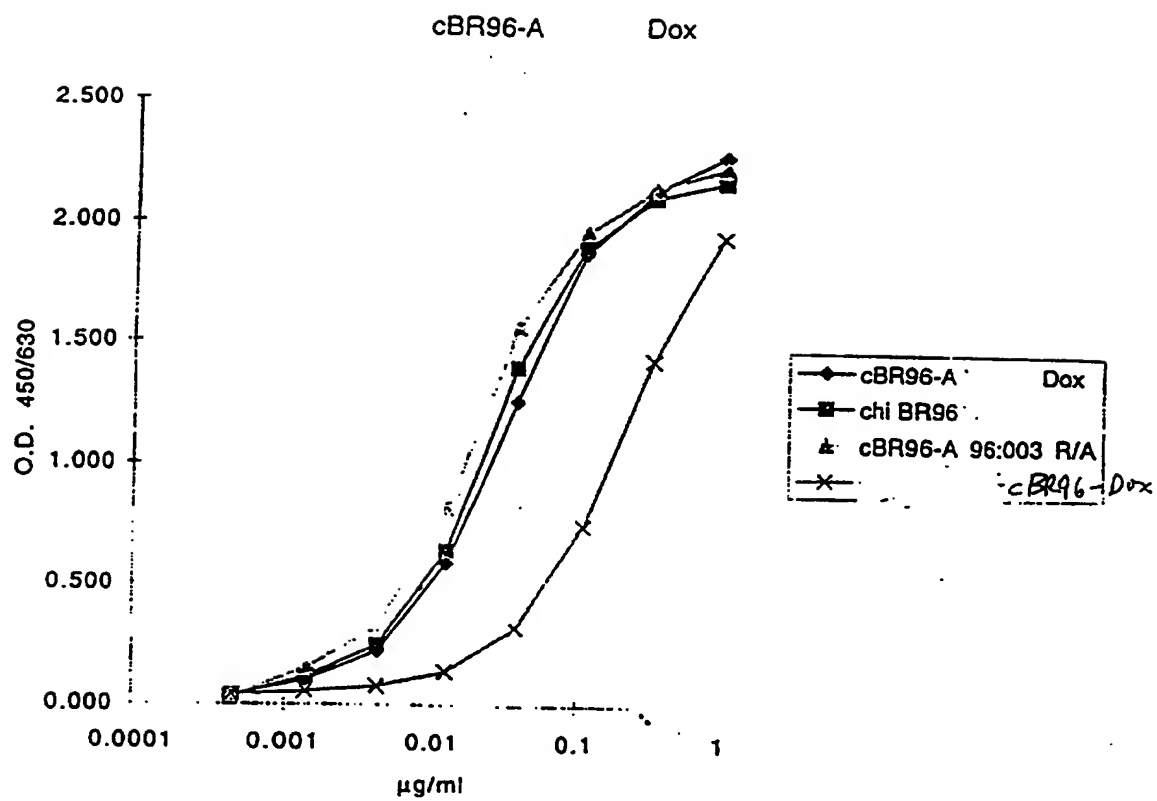
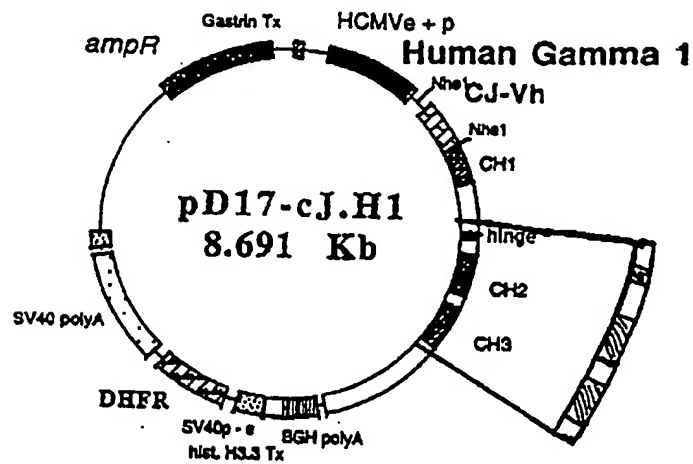


Figure 8



A- Hinge + CH<sub>1</sub> + CH<sub>3</sub> domains were removed from hR96 IgG1 construct by E.co <sup>-III</sup> restriction digestion .



B. 2 - Hinge + CH<sub>3</sub> domains amplified by PCR from L6 IgG1 construct lacking the CH<sub>2</sub> domain .

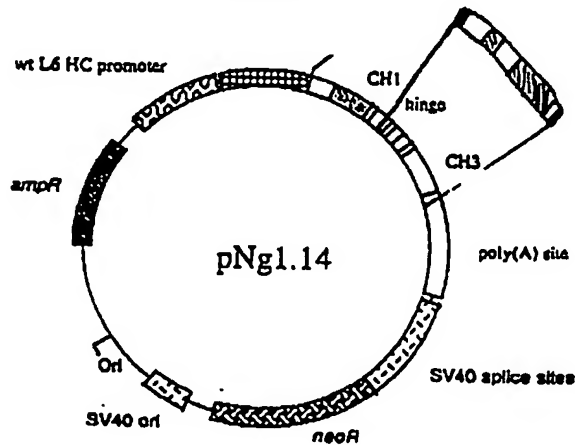


Figure 9

3 - Hinge +CH3 PCR fragment cloned by homologous recombination into E.co47-III site of BR96 IgG1 molecule.

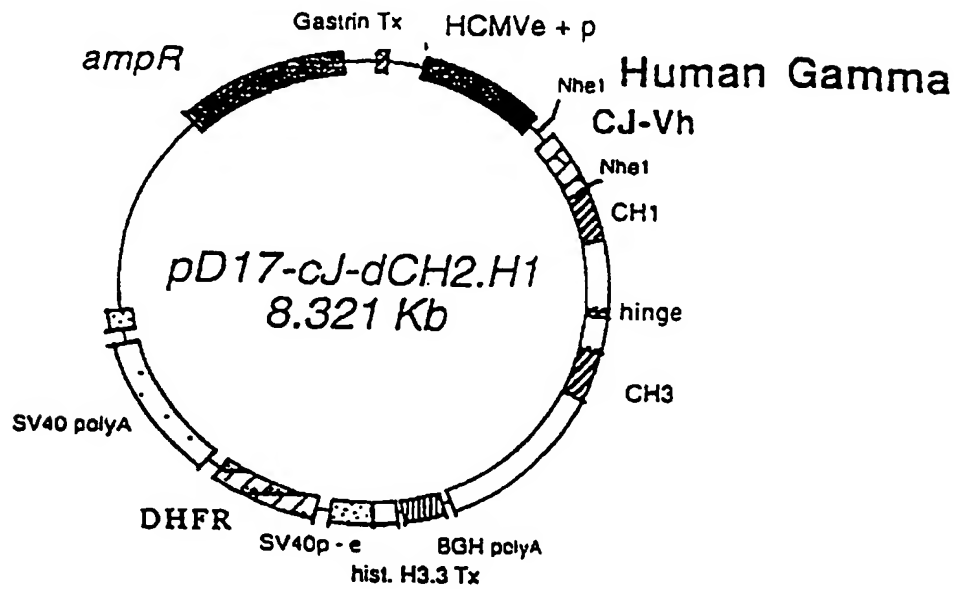
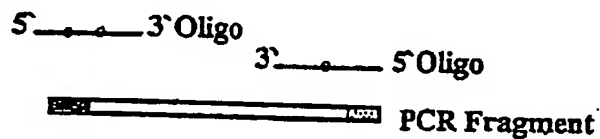


Figure 9

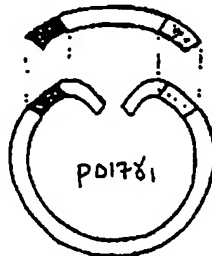
(CONTINUED)

**1- Introduction of mutations by site-directed mutagenesis on double-stranded plasmid DNA.**

**A- Mutations introduced into synthetic oligonucleotides used for the PCR amplification of CH2 domain.**



**B- Plasmid DNA linearized inside CH2 domain and co-transformed with PCR fragment into competent DH5 $\alpha$ .**



**C- Cloning mediated by homologous recombination yields transformants harbouring recombinant plasmids.**

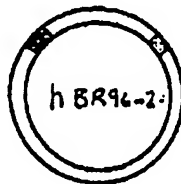
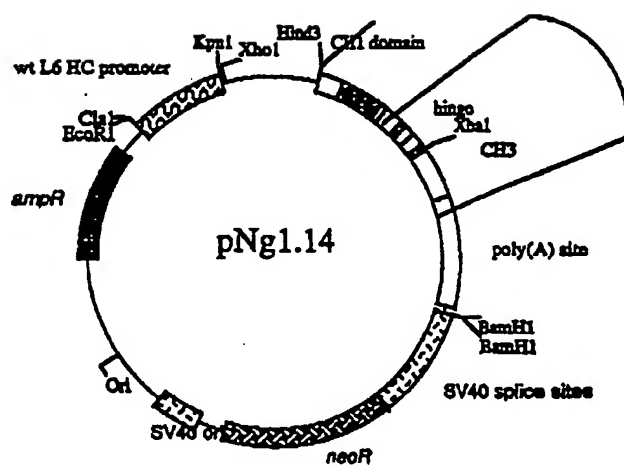


Figure 10

Figure 11



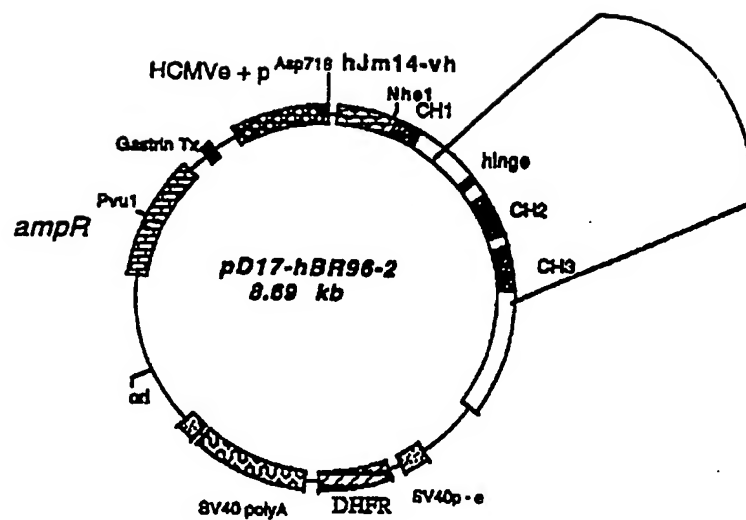


Figure 12

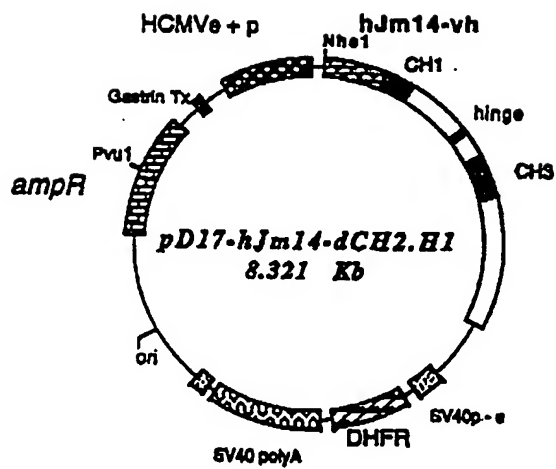


Figure 13



## pD17-cJ-dCH2.H1

10 GAGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCGG 40 50 60 70 80 90  
 CTGCTAGCC CTCTAGACGA TCCACTGGAC TCCGCGCGGC CGAGCTTAT CGGTCTCAT GGAAGAAAA CTTTTTTT TAATTTTAT TATTTTAT  
 100 TTTGAGATGG AGTTTGGCG CGATCTCCG ATCCCTATG GTGACTCTC AGTACAACT GCTCTGATG CCGATAGTTA AGCCAGTATC  
 AAACCTTACC TCAAAACCGG GCTAGAGGGC TAGGGGATAC CAGCTAGAG TCATGTTAGA CGAGACTAC GCGTATCAAT TCGGTCAATAG  
 190 200 210 220 230 240 250 260 270  
 TGCTCCCTGC TTGTGTGTG GAGTGGCTG AGTAGTGGC GAGCAAAAT TAAGCTACAA CAAAGCAAGG CTGACCGAC AATTGCATGA  
 ACGAGGACG AACACACAC CTCACGGAC CTCATCAGCG CTGCTTTTAA ATTGATGTT GTTCCGTTCC GAACTGGCTG TTAACGTACT  
 280 290 300 310 320 330 340 350 360  
 AGAATCTGCT TAGGGTTAGG CTTTTTGGC TGTTCGGCA TGTAAGGGC AGATATACGC GTTGACATTTG ATTATTGACT AGTTATTAT  
 TCTTAGACGA ATCCCAATCC GCAAAACCGG ACAGAGCGT ACATGCCCG TCTATATGCG CAATGTAAC TAATAACTGA TCAATAATTA  
 370 380 390 400 410 420 430 440 450  
 AGTAATCAAT TACGGGGTCA TTAGTTTCATA GCCCATATAT GGAGTTCCGC GTTACATTAAC TTACGGTAAA TGGCCCGCCT GGCTGACCGC  
 TCATTAGTTA ATGCCCCAGT AATCAAGTAT CCGGTATATA CCTCAAGCG CAATGTATTG AATGCCATTT ACCGGCGGA CCGACTGGCG  
 460 470 480 490 500 510 520 530 540  
 CCAACGACCC CCGCCCATTTG ACGTCAATAA TGACGTATGT TCCCATAGTA AGCCCAATAG GGACTTTCCA TTGACGTCAA TGGGTGGACT  
 GGTTCCTGGG GCGGGGTAAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TCGGGTTATC CCTGAAAGGT AACTGCAGTT ACCACCTGA  
 550 560 570 580 590 600 610 620 630  
 ATTTACGGTA AACTGCCAC TTGGCAGTAC ATCAAGTGA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT AAATGGCCCC  
 TAAATGCCAT TTGACGGGTG AACGGTCAAT TAGTTTCAAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA TTACCGGGC  
 640 650 660 670 680 690 700 710 720  
 CCTGGCATTA TGGCCAGTAC ATGACCTTAT GGAACCTTTC TACTTGGCAG TACATCTPAG TATTAGTCAT CGCTATTACC ATGGTATGC  
 GGACCGTAAT ACGGGTCATG TACTGGGATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC AATAACAGTA GCGATTAATGG TACCACATCG  
 730 740 750 760 770 780 790 800 810  
 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGA TTTCCNAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT  
 CCAAAACCGT CATGTAGTTA CCGCACCTTA TCGCCAACT GAGTGGCCCT AAAGTTTAC AGGTGGGTA ACTGCAGTTA CCTCAACA  
 820 830 840 850 860 870 880 890 900  
 TTTGGCACCA AATCAACGG GACTTTCCA AATGTCTGTA CAACTCGCC CCATTGACGC AATGGCGG TAGGCCGTGA CGGTGGGAGG  
 AAACCGTGGT TTTAGTTGCC CTGAAAGGTT TTACAGCAAT GTTGAGGCG GGTAACTGCG TTTACCGCC ATCCGACAT GCCACCTCC

Figure 14

15/56

## pD17-cJ-dCH2.H1

910 TCCTATTAAG CAGAGCTCTC TGGCTAACTA GAGAACCCAC TGCCTTACTGG CTTATCGAAA TTAATACGAC TCACTATAGG GACACCCCAAG  
 AGATATATTC GTCTCGAGAG ACGGATTGAT CTCCTGGGTG CCGAATGACC GAATAGCTTT ANTATGCTG AGTGATATCC CTCCTGGGTTC  
 1000 CTCTGGTACCA ATTTAAATG ATATCTCCTT AGGTCTCGAG TCTCTAGATA ACCGGTCAAT CGATTGGAAT TCTTGGGGCC GCTTGTCTAGC  
 GAACCATGGT TAAATTTAAC TATAGAGAA TCCAGAGCTC AGAGATCTAT TGGCCAGTTA GCTAACCTTA AGAACCCCG CGAACGATCG  
 1090 CACCATGGAG TTGTGGTAA GCTTGGTCTT TCTTGTCTCT TCTTTTAAAA GGTGTCCAGT GTGACGTAA TCTGTGGAG TCTGGGGGAG  
 GTGTACCTC AACACCAATT CGAACCHGA AGAACACGGA ACATAATTTT CCACAGGTCA CACTTCACTT AGACCACTC AGACCCCTC  
 1180 GCTTAGTCA GCCTGGAGGG TCCCTGAAAG TCTCTCTGTG AACCTCTGGA TTCACCTTCA GTGACTATTA CATGTATTGG GTTGGCCAGA  
 CGAATCAGT CGACCTCCC AGGACTTTC AGAGGACACA TTGGAGACCT AAGTGAAAGT CACTGATAAT GTACATAACC CAAGCGTCT  
 1270 CTCACAGAA GAGGCTGGAG TGGGTGCGAT ACATTAGTCA AGGTGGTAT ATAACCGACT ATCCAGACAC TGTAAGGGT CGATTACCA  
 GAGTCTCTT CTCGACCTC ACCCAGCGTA TGTAAATCAGT TCCACCACTA TATTGGCTGA TAGGTCTGTG ACATTTCCTA GCTAAGTGGT  
 1360 TCTCCAGAA CAATGCCAAG AACACCTGT ACCTGCAAT GAGCCGTCTG AAGTCTGAG ACACAGCCAT GTATTACTGT GCAGAGGCC  
 AGAGGTCTCT GTTACGGTTC TTGTGGACA TGGACGTTA CTGGCAGAC TTCAGACTCC TGTGTGGTA CATAATGACA CGTCTCCGG  
 1450 TGAACGAGG GGCCTGGTTT GCTTACTGG GCCAAGGNC TCTGTACAG TCTCTGTAG CTAGACCAAA GGGCCCATCG GTCTTCCCC  
 ACCTGCTGCC CCGACCAA CGAATGACCC CGGTTCCCTG AGACCACTG CAGAGACATC GATCCTGTT CCCGGTAGC CAGAACGGGG  
 1540 TGGCACCTC CTCGAAGAG ACCTCTGGG GCACAGGGC CTTGGCTGC CTTGGTCAAG ACTACTTCC CGAACCGGTG ACGGTGTCT  
 ACCGTGGAG GAGTCTCTG TGGAGACCC CGTGTGCGC CGACCGAGC GACCAGTTCC TGATGAAGG GCTTGGCCAC TGCACACGA  
 1630 GGAACCTCAG CGCCCTGACC AGCGCGTGC ACACCTTCCC GGCTGTCTTA CAGTCTCAG GACTCTACT CCTCAGCAG GTGGTCAACG  
 CCTTAGTCC CCGGACTTG TCGCCGACG TGTGGAAGG CCGACAGATC GTACAGATG GAGTCTGTG CACCACTGGC  
 1720 TGCCTCCAG CAGCTGGGC ACCCAGACT ACATCTGCA CGTGAATCAC AAGCCAGCA ACACCAAGT GGACAAGAA GTTGGTGAGA  
 ACGGAGGTC GTCGAACCC TGGTCTGGA TGTAGACGT GCACTTAGT TCCGGTCTG TGTGGTCCA CTTGTCTTT CAACCACTCT

Figure 14  
(continued)

16/56

## pD17-eJ-dCH2.H1

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1810 GGCAGCAC GGGAGGAGG GTCTCTCTG 1830 GAAGCCAGGC 1840 1850 1860 1870 1880 1890
CCGTCGTGT CCTCTCTCC CACAGACGAC CTTCCGTCCG AGTCCGAGG ACAGCCTCC GTAGGCGCG TACGTCGGG TCAGGTCCC
1900 AGCAAGCAG GCCCGTCTG CCTCTTCAAC CGGAGGCCCTC 1920 1930 1940 1950 1960 1970 1980
TCGTTCCGTC CCGGCGAGC GGAGAGTGG GCCTCCGGAG ACAGGCGGG TGAGTACGAG TCCCTCTCCC AGAGACCGA AAAGGGGTC
1990 GCTCTGGGA GGCACAGGT AGGTGCCCTT AACCCAGGCC 2010 2020 2030 2040 2050 2060 2070
CGAGACCCGT CCGTGTCCG TCCACGGGA TTGGGTCCG GACGTGTGT TCCCGGTCCA CGACCCGAGT CTGGAGCGTT CTCGGTATAG
2080 CGGAGGACC CTGCCCCGA CCTAAGCCCA CCCAAAGGC 2100 2110 2120 2130 2140 2150 2160
GCCCTCTCG GACGGGACT GGATTGGGT GGGGTTCCG CAACTCTCC ACTCCCTCAG CTCGGACACC TTCCTCTCTC CCAGATTCCA
2170 GTAACTCCA ATCTTCTC TGCAGAGCC AAATCTTGT 2190 2200 2210 2220 2230 2240 2250
CATTGAGGT TAGAAGAG ACCTCTCCG TTATAGACAC TGTTTGTAGT GTGTACGGT GTGTACGGT GGCACGGGTC CATTCGGTCG GGTCCGGAGC
2260 CCTTCCAGCT CAAGGGGGA CAGGTGCCCT AGAGTAGGCT 2280 2290 2300 2310 2320 2330 2340
GGAGGTCCA GTTCCGCCCT GTCCACGGGA TCTCATCCGA CGTAGGTCCC TGTTGTGTGC ACCCATGGTT GTACAGGCTT CCGTGTACCT
2350 CAGAGGCCG CTCGGCCAC CCTCTGCCCT GAGAGTGACC 2370 2380 2390 2400 2410 2420 2430
GTCTCCGGCC GAGCCGGTG GAGACGGGA CTCTCCTGG CGACATGGTT GGAGACAGGG ATGTCCCGTC GGGGCTCTTG CACAGGTGA
2440 CACCTGCCC CCATCCCGG ATGAGCTGAC CAAGAACCAG 2460 2470 2480 2490 2500 2510 2520
GTGGACGGG GTTAGGGCC TACTCGACT GTTCTTGTG CAGTCGGACT GCACGGACCA GTTTCGAG ATAGGTCGC TGTAGCGCA
2530 GAGGTGGAG AGCAATGGC AGCCGGAGAA CAACTACAG ACCACGCTC 2550 2560 2570 2580 2590 2600 2610
CCTCACCCTC TCGTTACCG TCGGCCCTCTT GTGATGTTT TGGTCCGGAG GGCACGACCT GAGGCTCCG AGGAAGAGG AGATGTCGT
2620 GCTCACCGT GACAGAGCA GGTGGCAGCA GGGGAACCTC 2640 2650 2660 2670 2680 2690 2700
CGAGTGGCAC CTGTTCTGCT CCACGCTGCT CCGTCTGCTTCTCTATGCT CCGTGTATGCA TGAGGCTCTG CACAACCACT ACACGAGAA
CGAGTGGCAC CTGTTCTGCT CCACGCTGCT CCGTCTGCTTCTCTATGCT CCGTGTATGCA TGAGGCTCTG CACAACCACT ACACGAGAA
CGAGTGGCAC CTGTTCTGCT CCACGCTGCT CCGTCTGCTTCTCTATGCT CCGTGTATGCA TGAGGCTCTG CACAACCACT ACACGAGAA

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Figure 14  
(continued)

17/26

## pD17-cJ-dCH2.H1

2710 GAGCCTCTCC CTGTCTCCGG GTAAATGAGT GCGACGGCTG GCGAGCCCTC GCGAGCCCTC GCGAGCCCTC GCGAGCCCTC GCGAGCCCTC GCGAGCCCTC  
 CTCGGAGAGG GACAGAGGCC CATTTACTCA CGCTGCCGCG CGTTCCGGGG CGAGGGGCCC CGAGGGGCCC CGAGGGGCCC CGAGGGGCCC CGAGGGGCCC  
 2800 GTACCCCTCTG TACATACTTC CCGGGCGGCC AGCATGGAAA TAAAGCACCC AGCGCTGCCC TCGGACCGGG TCGGACCGGG TCGGACCGGG TCGGACCGGG  
 CATGGGGGAC ATGTATGAAG GCGCCGCGGG TCGTACCTTT ATTTCTGTGG TCGGACCGGG TCGGACCGGG TCGGACCGGG TCGGACCGGG TCGGACCGGG  
 2890 CCACGGGTCA GCGCGAGTCT GAGGCCCTGAG TGGCATGAGG GAGGCGAGAG GGGTCCCACT GTTCCCACT GTTCCCACT GTTCCCACT GTTCCCACT  
 GGTGCCCACT CCGGCTCAGA CTCGGACTC ACCGTACTCC CTCCTGTCTG CCGAGGTGA CCGAGGTGA CCGAGGTGA CCGAGGTGA CCGAGGTGA  
 2980 TGCCTGGGCC CCTTAGGGTG GGGCTCAGCC AGGGGCTGCC CTGCGGAGGG TGGGGGATTT GCCAGCGTGG CCCTCCCTCC AGCAGCACCT AGCAGCACCT  
 ACGACCCCG GGGATCCAC CCGGATCCG TCCCGGACGG GAGCGGTCCC ACCCCCTAAA CGGTCCGACC GGGAGGGAGG TCGTCTGTGA TCGTCTGTGA  
 3070 GCGCTGGGCT GGGCCACCGG AAGCCCTAGG AGCCCTGCGG GACAGACACA CAGCCCTGCG CTCTGTAGGA GACTGTCTG TTCTGTGAGC TTCTGTGAGC  
 CCGGACCCGA CCGGTGCCC TTGGGATCC TTGGGATCC TTGGGATCC TTGGGATCC TTGGGATCC TTGGGATCC TTGGGATCC TTGGGATCC TTGGGATCC  
 3160 GCGCTGGGCT TCCCGACTC CATGCCACT CCGGGGCACT CCGGGGCACT CCGGGGCACT CCGGGGCACT CCGGGGCACT CCGGGGCACT CCGGGGCACT  
 CCGGGACAGG AGGGCTGGAG GTACGGGTGA GCGCCCTGAC GCGCCCTGAC GCGCCCTGAC GCGCCCTGAC GCGCCCTGAC GCGCCCTGAC GCGCCCTGAC  
 3250 GGCCTAACCC CCTGGGCTGCC CTGCCCCAGCC TCGCACCCGC AGCGTGGCG TACCCCTGAG TTGGCTGAG TTGGCTGAG TTGGCTGAG TTGGCTGAG  
 CCTGATTTGG GGACCGACCG GACGGGTGCG GACGGGTGCG GACGGGTGCG GACGGGTGCG GACGGGTGCG GACGGGTGCG GACGGGTGCG GACGGGTGCG  
 3340 GACTGGTGA GATGCCACCA CACACACTCA GCGCAGACCC GTTCAACAAA CCGCCGACTG AGGTTGGCGG GCCACACGGC CACACACAGC CACACACAGC  
 CTGACCACTG CTACGGGTGT GTGTGTGAGT CCGGTCTGGG CAAGTTGTTT GGGGCTGAC TCCAACCCGG CCGTGTGCGG GTGTGTGAGT GTGTGTGAGT  
 3430 ACAGGTGAC GCGTCAACA CCGAGCTTCA CCGGGGCGAA CTGCACAGCA CCCGAGCCAG AGCAAGGTCC TCGCACACGT GAACACTCCT GAACACTCCT  
 TGTGCACGT CCGAGTGTGT GCGTGGAGT GCGCCGCTT GCGCCGCTT GCGCCGCTT GCGCCGCTT GCGCCGCTT GCGCCGCTT GCGCCGCTT GCGCCGCTT  
 3520 CCGACACAGG CCGCCACAGG CCGCCACAGG CACCTCAGG CCGCCACAGG CCGCCACAGG CCGCCACAGG CCGCCACAGG CCGCCACAGG CCGCCACAGG  
 GCTGTGTGTC GCGGGTCTC GCGGGTCTC GCGGGTCTC GCGGGTCTC GCGGGTCTC GCGGGTCTC GCGGGTCTC GCGGGTCTC GCGGGTCTC

Figure 14  
(continued)

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## pD17-cJ-dCH2.H1

3610 CCAGCCCTCC TCTCACAAGG 3620 GTGCCCTGC AGCGCCACA 3640 CACACACAGG 3650 GGATCACACA 3660 CCACGTCACG 3670 TCCCTGGCCC 3680 TGGCCCACTT 3690  
 GGTCCGAGG AGAGTGTTC CACGGGACG TCGGCGGTGT GTGTGTGTCC CCTAGTGTGT GGTGCAGTGC AGGACCCGG ACCGGGTGAA  
 3700 CCCAGTCCG CCTTCCCTG CAGGACGGAT CAGCCTCGAC 3730 3740 3750 3760 3770 3780  
 GGTACACGC GGGAGGGAC GTCTGCCTA GTCCGCTGAC GTCCGAGCTG ACACGGAGA TCACGGTGC GTAGACAACA AACGGGAGG GGCACCGAA  
 3790 CCTTGACCT GGAAGGTGC ACTCCACTG TCCCTTCCCTA ATAAATGAG GAAATGTCAT CGCATGTGTCT GAGTAGGTCT CATTCATATC 3870  
 GGAACCTGGG CCTTCCACG TGAGGTGAC AGGAAGGAT TATTTTACTC CTTTAACTGTA GCGTAACAGA CTCATCCACA GTAAGATTAAG  
 3880 TGGGGGTGG GGTGGGGCAG CACACGACG GAGGAGATTG 3910 3920 3930 3940 3950 3960  
 ACCCCACCC CCACCCGTC CTGTGTGTC CCTCTTAAC CTTCTGTGTA TCGTCCGTAC GACCCCTAGC CTGGGGCTCT ATGGCTTCTG  
 3970 AGCGGAAAG AACCACTGG GGTCTTAGGG 4000 4010 4020 4030 4040 4050  
 TCCGCCCTTC TTGGTCGACC CCGAGATCC CCATAGGGT GCGCGGACA TCGCCCGTA ATTCCGCGC CCCACACCAC CAATGCCGT  
 4060 GGTGACGC TACACTTGC AGCGCCCTAG CGCCGCTCC TTTCCCTTC TTTCTGCCAC GTTCGCCGGG CCTCTCAAAA 4140  
 CCGACTGCG ATGTGAACG TCCCGGATC GCGGCGAGG AAGCGAAG AAGAGCGGTG CNAAGCGGCC GGAGAGTTTT  
 4150 AAGGGAAAA AAGCATGCAT CTCATTTAGT CAGCAACCAT AGTCCGCCC CTAACCTCCG CCTAACTCCG CCGAGTTCCG 4230  
 TTCCCTTTT TCGTAGGTA GAGTTAATCA GTCGTGGTA TCAGGCGCG GATTGAGCG GGTAGGGCGG GGTAGAGGC GGTCACAGGC  
 4240 CCCATTCTC GCGCCATGGC TGACTAATTT TTTTATTTA TGCAGAGGCC GAGGCGCCT CCGCTCTGA GCTATTCCAG AAGTAGTGAG 4320  
 GGTAAAGAG CCGGTACCG ACTGATTAA AAAATAAAT AGTCTCCG CTTCCGCGGA CCGGAGACT CGATAAGGTC TTCATCACTC  
 4330 GAGGCTTTT TGGAGCCCTA GGCTTTTGA AAAAGCTGG ACAGCTCAG GCTCGGATTT CGCGCCAAAC TTGACGGCAA TCCTAGCGTG 4410  
 CTCGAAAA ACCTCGGAT CCGAAACGT TTTTCGAAC TGTCAGTCC CGACGTAAG GCGCGGTTG AACTGCCGT AGGATCCGAC  
 4420 AAGGCTGTA GGAATTTAT CCGCGTCCA TCATGGTCC ACCATGAAC TGCATCTCG CCGTGTCCA AAATATGGG ATTGGCAAGA 4500  
 TTCCGACCAT CCTAATAAT GGGCAGCGT AGTACCAGC TGGTAATG ACCTAGACG GGCACAGGT TTTATACCC TAACCGTTCT

Figure 14  
(continued)

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pD17-cl-dCH2.H1

4510 ACGGAGACCT ACCCTGGCCT CGGCTCAGGA ACGAGTTCAA GACTTCCAA AGATGACCA CAACTCTTC AGTGGAGGT AAACAGAAATC 4590  
 TGCCTCTGGA TGGGACCGGA GCGGAGTCTT TGCTCAAGTT CATGAGGTT TCTTACTGGT GTTGGAGAG TCACCTTCCA TTGTCTTTAG  
 4600 TGGTGAATTAT GGGTAGGAAA ACCTGGTTCT CCAJTCCTGA GAAGATCGA CCTTTAAGG ACAGAAATTAA TATAGTTCTC AGTAGAGAAC 4680  
 ACCACTAATA CCCATCCTTT TGGACCAAGA GGTAAAGACT CTCTTAGCT GGAAATTTCC TGCTTAATT ATATCAAGAG TCATCTCTTG  
 4690 TCAAAGAAC ACCACGAGGA GCTCATTTTC TTGCCAAAAG TTGGGATGAT GCCTTAAGAC TTATTGAACA ACCGGAATTG GCAAGTAAAG 4770  
 AGTTTCTTGG TGGTGTCTCT CGAGTAAAG AACGTTTTC AAACCTACTA CCGNATTTCTG AATAACTTGT TGGCCTTAAC CGTTCAATTC  
 4780 TAGACATGCT TTGGATAGTC GGAGGCAGTT CTGTTTACCA GGAAGCCATG AATCAACACG GCCACCTTAG ACTCTTTGTG ACAAGGATCA 4860  
 ATCTGTACCA AACCTATCAG CCTCCGTCAA GACAAATGGT CCTTCGGTAC TTAGTTGGTC CGGTGAATC TGAGAAACAC TGTTCTTAGT  
 4870 TGCAGGAATT TGAAGTGAC ACGTTTTC CAGAAATGA TTGGGAAA TATAACTTC TCCAGAATA CCCAGCGTC CTCCTGAGG 4950  
 ACGTCTTAA ACTTTCAC TGCAAAAGG GTCTTAACT AAACCCCTTT ATATTGAAG AGGTCTTAT GGTCCGCAG GAGAGACTCC  
 4960 TCCAGGAGGA AAAAGGCATC AAGTATAAGT TTGAAGTCTA CGAGAAGAA GACTAACAGG AAGTGTCTT CAAGTTCTCT GCTCCCTCC 5040  
 AGGTCTCTCT TTTTCCGTAG TTCAATATCA AACTTCAGAT GCTCTTCTTT CTGATTTGCC TTCTACGAAA GTTCAGAGA CGAGGGGAGG  
 5050 TAAAGCTATG CATTTTATATA AGACCATGGG ACTTTTGCTG GCTTAGATC TCTTTGTAA GGAACCTTAC TTCTGTGGTG TGACATAATT 5130  
 ATTTGGATAC GTAAAAATAT TCTGGTACCC TGAACACGAC CGAATCTAG AGAAACACTT CCTTGAATG AAGACACCAC ACTGTATTAA  
 5140 GGACAACTA CCTACAGAGA TTTAAAGTC TAAGGTAAAT ATAAATTTT TAAGTGTATA ATGTGTTAA CTACTGATTC TAATGTTTG 5220  
 CCTGTGTGAT GGATGTCTCT AAATTTGAG ATTCCATTTA TATTTTAAA ATTCACATAT TACACAAATT GATGACTAAG ATTAAACAAAC  
 5230 TGTATTTTAG ATTCCAACCT ATGGAACGTA TGAATGGAG CAGTGGTGA ATGCCTTTAA TGAGGAAAC CTGTTTTGCT CAGAGAAAT 5310  
 ACATAAAAATC TAAGGTGGA TACCTTGACT ACTTACCCTC GTACCCACT TACGGAATTT ACTCCTTTG GACAAAACGA GTCTTCTTTA  
 5320 GCCATCTAGT GATGATGAGG CTACTGCTGA CTCTCAACAT TCTACTCTC CAAAAAGAA GAGAAAGGA GAAGACCCA AGGACTTTCC 5400  
 CGGTAGATCA CTACTACTCC GATGACGACT GAGAGTTGTA AGATGAGGAG GTTTTTTCTT CTCTTCCAT CTTCGGGGT TCCTGAAAGG

Figure 14  
(continued)

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## pD17-cJ-dCH2.H1

5410 TTCAGAAATG CTAAGTTTTT TGAGTCATGC 5430 5440 5450 5460 5470 5480 5490  
 AAGTCTTAAC GATTCAAAA ACTCAGTAGC ACACAATCA TTATCTTGAG AACGAACGA TGGTGTTC TGGTATTTAC ACCACAAGG AAAAGCTGC  
 5500 ACTGCTATAC AAGAAATTA TGGAAATAA TTCTGTAAAC 5520 5530 5540 5550 5560 5570 5580  
 TGACGATATG TTCTTTAAT ACCTTTTAT AAGACATTGG AATATTTCAT CGGTATTGTC AATATTAGTA TTGTATGACA AAAAGAATG  
 5590 5600 5610 5620 5630 5640 5650 5660 5670  
 TCCACACAG CATAGAGTGT CTGCTATTAA TAACTATGCT CAATAATGT GTACTTTAG CTTTAAAT TTATAAGGG TTATAAGGA  
 AGGTGTGTC GTATCTACA GACGATAAT ATTGATACGA GTTTTAAACA CATGGAATC GAATAATTAA ACATTTCCCC AATTATCTCT  
 5680 5690 5700 5710 5720 5730 5740 5750 5760  
 ATATTTGATG TATAGTGCCT TGACTAGAGA TCATAATCAG CCATACCACA TTTGTAGAGG TTTTACTTCC TTATAAAAC CTCCCACACC  
 TATNAACTAC ATATCACGGA ACTGATCTCT AGTATTAGTC GGTATGGTGT AATCATCTCC AATATGAACG AATTTTTT GAGGGTGTGG  
 5770 5780 5790 5800 5810 5820 5830 5840 5850  
 TCCGCTGAA CCTGAACAT AAATGAATG CAATGTGTGT TGTAACTTG TTTATTGCGAG CTTATAATGG TTACAATAA AGCAATAGCA  
 AGGGGACTT GGACTTTGTA TTTTACTTAC GTTAACAACA ACAATTGAAC AATAACGTC GAATATTACC AATGTTTATT TCGTTATCGT  
 5860 5870 5880 5890 5900 5910 5920 5930 5940  
 TCACAAATTT CACAAATAA GCATTTTTTT CACTGCATTC TAGTGTGGT TTGTCCAAC TCATCAATGT ATCTTATCAT GTCTOGATCG  
 AGTGTTTAAA GTGTTTATTT CGTAAATAA GTGACGTAA GTCAACACCA AACAGGTTTG AGTAGTTACA TAGAATAGTA CAGACCTAGC  
 5950 5960 5970 5980 5990 6000 6010 6020 6030  
 GCTGGATGAT CCTCCAGCG GGGATCTCA TGCTGGAGTT CTTCCGCCAC CCCAACTTGT TTATTGCGAG TTATAATGT TACAATAA  
 CGACCTACTA GGAGGTGCG CCCGTAGAGT ACGACCTCAA GAAGCGGGTG GGGTTGAACA AATAACGTCG AATATTACCA ATGTTTATTT  
 6040 6050 6060 6070 6080 6090 6100 6110 6120  
 GCAATAGCAT CACAAATTC ACAATNAAG CATTTTTTC ACTGCATCT AGTGTGGGT TGTCCAACCT CATCAATGTA TCTTATCATG  
 CGTTATCGTA GTGTTTAAG TGTTTATTTC GTAAAAAAG TGACGTNAGA TCACACCAA ACAGGTTTGA GTAGTTACAT AGAATAGTAC  
 6130 6140 6150 6160 6170 6180 6190 6200 6210  
 TCTGTATACC GTCGACCTCT AGCTAGAGCT TGGGCTAATC ATGCTCATAG CTGTTTCCG TGTGAATG TTATCCGTC ACAATTCCAC  
 AGACATATGG CAGCTGGAGA TCGATCTCCA ACCGATTAG TACCAGTATC GACAAAGGAC ACACCTTAAAC AATAGGCGAG TGTAAAGGTG  
 6220 6230 6240 6250 6260 6270 6280 6290 6300  
 ACAACATAG AGCCGAGC ATAAAGTGA AAGCTGGG TGCTAAATGA GTGAGCTAAC TCACATTAAT TCGTTCGCG TCACATCCCG  
 TGTGTATGC TCGGCTTCG TATTCACAT TTCCGACCCC ACCGATTACT CACTCGATG AGTGAATTA AGCAAGCG AGTAGGGCG

Figure 14  
(continued)

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## pD17-cJ-dCH2.H1

6310 CTTTCAGTC GGGAAACCTG TCGTGCCAGC 6320 6330 6340 6350 6360 6370 6380 6390  
 GAAAGTCAG CCTTTGGAC AGCACGGTCG ACCTAATTAC TTAGCCGGTT GCAGCCCTT CTTCCGCCAA CCATATACCC GCGAGAAGGC  
 6400 CTTCTCGCT CACTGACTCG CTGCGCTCGG 6410 6420 6430 6440 6450 6460 6470 6480  
 GAAAGAGCGA GTGACTGAGC GACCGAGCC GACCAAGCCGA TCGTTGGCT GCGCGAGCGG GTATCAGCTC TGAGTTTCGG CCATATATGCC AATAGGTGTC  
 6490 6500 6510 6520 6530 6540 6550 6560 6570  
 AATCAGGGGA TAACGCAGGA AAGAACATGT GAGCAAAAGG CCAGCAAAAG GTCGCTTTTC CCGTTCGCTG CGGTTTTC CCGTTTTC  
 TTAGTCCCTT ATTGCTCCT TTCTTGATCA CTGCTTTTC GGTCTTTTC CCGTCTTTC GCGCAACGAC CCATATATTC ATGCTCCGCA  
 6580 6590 6600 6610 6620 6630 6640 6650 6660  
 ATAGGCTCG CCCCCTGAC GAGCATCACA AAAAACTGAG CTCAAGTCAG AGGTGGGAA ACCCGACAGG ACTATATAAGA TACCAGGCGT  
 TATCCGAGGC GGGGGAATG CTCTAGTGT TTTTAGCTGC GAGTTGAGTC TCCACCGCTT TGGGCTGTC TGATATTTCT ATGCTCCGCA  
 6670 6680 6690 6700 6710 6720 6730 6740 6750  
 TTCCCTCTG AAGCTCCCTC CTGTTCCGAC CTTGCTCGCTT ACCGATTAOC TGGGCTATCG ACAGCGGAA AGAGGGAAGC CCTTCGACCC  
 AAGGGGACC TTCGAGGGAG CACCGAGAG GACAAAGCTG GACAGGCGAA TGGCTATCG ACCCGACACA CGTCTTCGG GCGCAAGTCG  
 6760 6770 6780 6790 6800 6810 6820 6830 6840  
 CGCTTTCTCA ATGCTCAGC TGTAGGTATC TCAGTTGGT GTAGGTCTGT CCGTCCNAGC TGGGCTGTGT GCACGAAACC CCGTTTCAGC  
 GCGAAGAGT TACGAGTGC ACATCCATAG AGTCAAGCCA CATCCAGCAA GCGAGGTTCG ACCCGACACA CGTCTTCGG GCGCAAGTCG  
 6850 6860 6870 6880 6890 6900 6910 6920 6930  
 CCGACCGCTG CGCTTATCC GGTAACTATC GTCTTGAGTC CAACCGGTA AGACACGACT TATCCGCACT TATCCGCTGA ATAGCGGTGA CCCTCTCTCG TGACCATGT  
 GCTTGGCAG CCGGAATAG CCAATTGATAG CAGAACTCAG GTTGGGCCAT TCTGTGCTGA ATAGCGGTGA CCCTCTCTCG TGACCATGT  
 6940 6950 6960 6970 6980 6990 7000 7010 7020  
 GGATTAGCAG ACGAGGTAT GTAGGCGGTG CTACAGAGTT CTGGAAGTGG TGGCTAACT ACGCTACAC ACCTAAGGACA GTATTTGGTA  
 CCTAATCTC TCGTCCATA CATCCGCCAC GATGTCCTAA GAATTCACC ACCGATTTGA TGGCATGTG ATCTTCTCTG CATTAACCAT  
 7030 7040 7050 7060 7070 7080 7090 7100 7110  
 TCTGCGCTCT GCTGAAGCCA GTTACTCTG GAAAAAGAT TGTAGCTGT TGTAGCTGT TGTAGCTGT TGTAGCTGT TGTAGCTGT  
 AGACGCGAGA CGACTTCGGT CAATGGAAGC CTTTTTCTCA ACCATGAGA ACTAGGCGCT TGTAGCTGT TGTAGCTGT TGTAGCTGT  
 7120 7130 7140 7150 7160 7170 7180 7190 7200  
 TTGTTTGCAA GCAGCAGATT ACCCGAGAA AAAAGGATC TCAAGAGAT CCTTTGATCT TTTCTACGCG GTCTGACGCT CAGTGGAAAG  
 AACAAACGTT CCGCTCTAA TGGGCTCTT TTTTCTCTAG AGTTCTCTA GGAAGTCTA AAGATGCC CAGACTGCA GTACCTTTCG

Figure 14  
(continued)

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7210	7220	7230	7240	7250	7260	7270	7280	7290
AAACTCAG	TTAAGGAT	TTGGTCAT	GATTATCA	AAGGATCT	ACCTAGAT	TTTAAATTA	AAAATGA	TTTAAATCA
TTTTCAGT	CAATCCCT	AAACAGTA	CTAATAGT	TTCTAGAG	TGATCTAG	AAATTTAA	TTTACTCA	AAATTTAGT
7300	7310	7320	7330	7340	7350	7360	7370	7380
TCTAAGTA	ATATGAGTA	ACTTGGTC	ACAGTTAC	ATGCTTAAT	AGTGAGGC	CTATCTCAG	GATCTGTCT	TTTCTGTTAT
AGATTTCA	TATACTCAT	TGAAACAG	TGTAATGCT	TACGAATTA	TCACTCCGT	GATAGAGTC	CTAGACAGAT	AAAGCAAGTA
7390	7400	7410	7420	7430	7440	7450	7460	7470
CCATAGTGC	CTGACTCC	GTCTGTAG	TAACTACG	ACGGAGGC	TTACCATCT	GCCCCAGTC	TGCAATGAT	CCGCGAGAC
GGTATCAAC	GACTGAGGG	CAGCACAT	ATTGATGCT	TGCCCTCC	ATGTGTAG	CGGGGTCA	ACGTTACTAT	GGCGCTCTGG
7480	7490	7500	7510	7520	7530	7540	7550	7560
CACGCTCAC	GGCTCCAG	TTATCAGCA	TAAACAGC	AGCGGAGG	GCGGAGCG	GAAGTGTCC	TGCAACTTTA	TCCGCTCTCA
GTGCGAGTG	CCGAGGTCT	AAATAGTCT	ATTGTGCTG	TCCGCTTCC	CGGCTCGCT	CTTCACCAG	ACGTTCAAT	AGGCGGAGT
7570	7580	7590	7600	7610	7620	7630	7640	7650
TCCAGTCTAT	TAAATGTTGC	CGGAAGCTA	GAGTAAAGT	TTGCGCAGT	AAATAGTTGC	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG
AGGTCAGATA	ATTAAACAG	GCCCTTCAT	CTCATTCAT	AACGGTCA	TTATCAAA	CGTTGCAAC	ACGTTAACA	TGTCCTGAGC
7660	7670	7680	7690	7700	7710	7720	7730	7740
TGGTGTACG	CTGCTCGTT	GGTATGCTT	CATTACGCT	CGGTTCCTA	CGATCAAGC	GAGTTACAT	ATCCCCCAT	TTGTGCAAAA
ACCACAGTC	GACGAGCAA	CCATACCGA	GTAAGTCTG	GCCAAGGTT	GCTAGTTCC	CTCAATGTAC	TAGGGGTAC	AACAGTTTTT
7750	7760	7770	7780	7790	7800	7810	7820	7830
AAGCGTTAG	CTCCTTCGT	CTCCGATCG	TTGTCAGAG	TAAAGTTG	GCAGTGTAT	CACATATGG	TATGGCAGCA	CTGCATAAT
TTCCCCATC	GAGGAGCCA	GGAGGCTAG	AACAGTCTC	ATTCACCG	CGTCAACAT	GTAAGTACCA	ATACCGTCT	GACGTATTAA
7840	7850	7860	7870	7880	7890	7900	7910	7920
CTCTTACTGT	CATGCCATC	GTAAGATCT	TTCTGTGAC	TGGTGAATP	TCAACCAAG	CATTCTCAG	ATAGTGTATG	CGGCGACCGA
GAGATGACA	GTACGGTAG	CATTCTACA	AAAGACACT	ACCACATG	AGTTGGTCA	GTAAGACTCT	TATCACATAC	GGCGCTGGCT
7930	7940	7950	7960	7970	7980	7990	8000	8010
GTTCCTCTTG	CCCGGGTCA	ATACGGGAT	ATACGGGCC	ACATAGAGA	ACTTTAAAG	TGCTCATAT	TGGAAACGT	TCTTCGGGGC
CAACGAGAC	GGCGCGCAG	TATGCCCTAT	TATGGCGCG	TGTAATCTCT	TGAAATTTT	ACGAGTAGTA	ACCTTTTGA	AGAAGCCCCG
8020	8030	8040	8050	8060	8070	8080	8090	8100
GAAACTCTC	AAGGATCTTA	CGCTGTGTA	GATCCAGTT	GATGTACCC	ACTCGTGCAC	CCAACTGATC	TTACAGCATCT	TTTACTTTTCA
CTTTGTAGAG	TTCTTAGNAT	GGCGACACT	CTAGGTCAAG	CTACATTTGG	TGAGCAGCTG	GGTTGACTAG	AAGTCTGAGA	AAATGAAAGT

Figure 14  
(continued)

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```
8110      8120      8130      8140      8150      8160      8170      8180      8190
CCAGCGTTTC TGGGTGAGCA AAAACAGGAA GGC AAAATGC CGCAAAAAG GGAATNAGGG CGACACGGA ATGTTGAATA CTCATCTCT
GGTCGCAAG ACCCACTCGT TTTGTGCTT CCGTTTACG GCGTTTTC CCTATTCCC GCTGTGCTT TACNACTTAT GAGTATGAGA

8200      8210      8220      8230      8240      8250      8260      8270      8280
TCCTTTTCA ATATTATTGA AGCATTATC AGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT AAACAATAG
AGGAAAAAGT TATAATACT TCGTAATACT TCCCAATAC AGAGTACTCG CCTATGTATA AACTTACATA AATCTTTTA TTTGTTTATC

8290      8300      8310      8320      8330
GGGTTCCGG CACATTTCCC CGAAAAGTGC CACCTGAGGT C
CCCAAGGCG GTGTAAAGGG GCTTTTCACG GTGGACTGCA G
```

Figure 14  
(continued)

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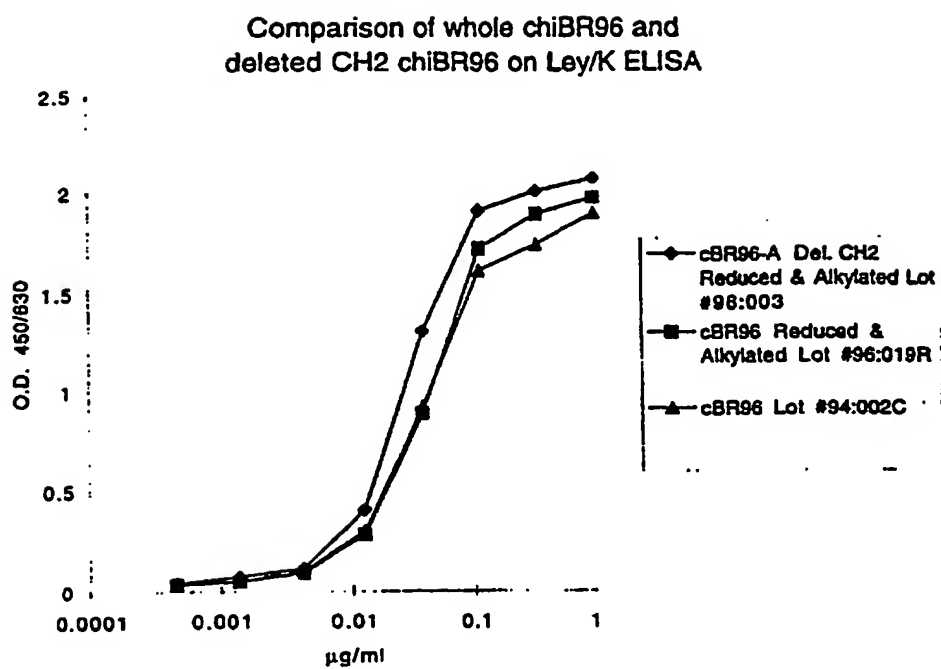


Figure 15

hBR96-2B: L235 to A235 and G237 to A237

hBR96-2C: E318 to S318, K320 to S320, and K322 to S322

hBR96-2D: P331 to A331

hBR96-2E: L235 to A235, G237 to A237, E318 to S318, K320 to S320, and K322 to S322

hBR96-2F: L235 to A235, G237 to A237, and P331 to A331

hBR96-2G: E318 to S318, K320 to S320, K322 to S322, and P331 to A331

hBR96-2H: L235 to A235, G237 to A237, E318 to S318, K320 to S320, K322 to S322, and P331 to A331

Figure 16

FIGURE 17

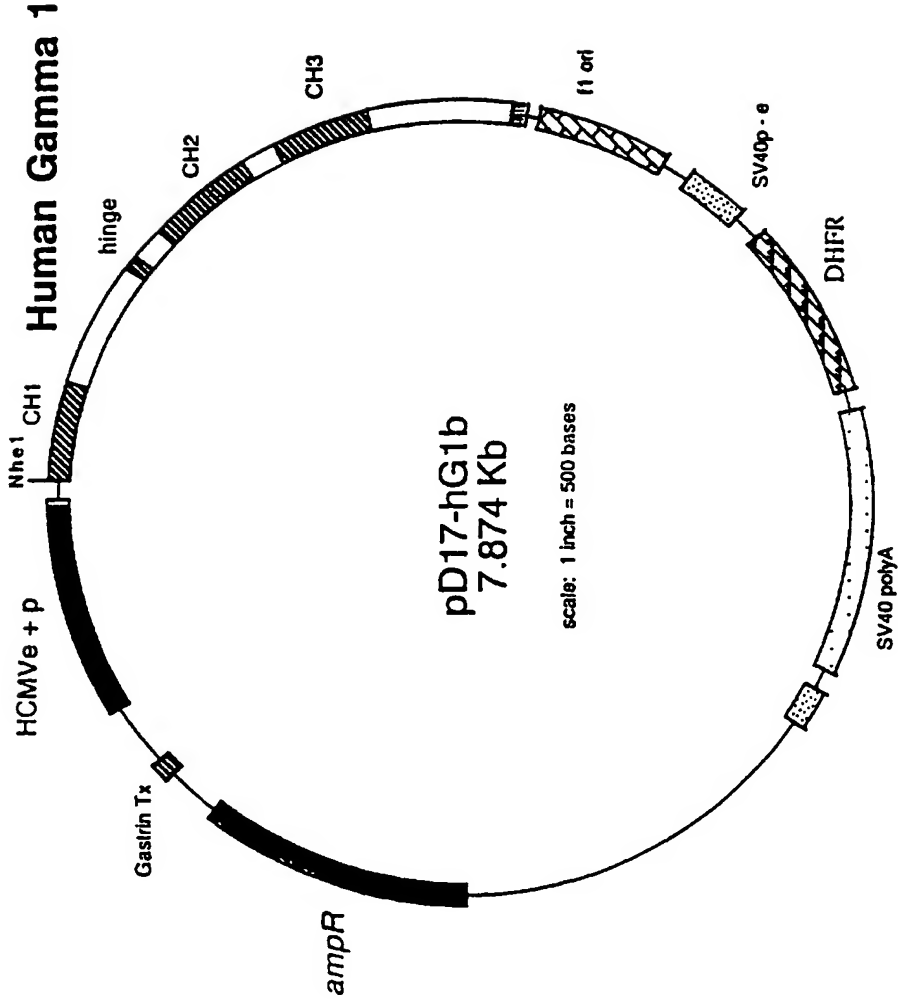


FIGURE 18A

1 GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC  
51 GGTCAATCGA TTGGAATTCT TGCGGCCGCT TGCTAGCCAC CATGGAGTTG  
101 TGGTTAAGCT TGGTCTTCCT TGTCCTTGTT TTAAAAGGTG TCCAGTGTGA  
151 AGTGCAACTG GTGGAGTCTG GGGGAGGCTT AGTGCAGCCT GGAGGGTCCC  
201 TGCGACTTTC CTGTGCTGCA TCTGGATTCC CGTTCAGTGA CTATTACATG  
251 TATTGGGTTC GCCAGGCTCC AGGCAAGGGA CTGGAGTGGG TCTCATACAT  
301 TAGTCAAGAT GGTGATATAA CCGACTATGC AGACTCCGTA AAGGGTCGAT  
351 TCACCATCTC CAGAGACAAT GCAAAGAACA GCCTGTACCT GCAAATGAAC  
401 AGCCTGAGGG ACGAGGACAC AGCCGTGTAT TACTGTGCAA GAGGCCTGGC  
451 GGACGGGGCC TGGTTTGCTT ACTGGGGCCA AGGGACTCTG GTCACGGTCT  
501 CTTCCGCTAG CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC  
551 AAGAGCACCT CTGGGGGCAC AGCGGCCCTG GGCTGCCTGG TCAAGGACTA  
601 CTTCCCCGAA CCGGTGACGG TGTCGTGGA CTCAGGCGCC CTGACCAGCG  
651 GCGTGACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT CTACTCCCTC  
701 AGCAGCGTGG TCACCGTGCC CTCCAGCAGC TTGGGCACCC AGACCTACAT  
751 CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTGGAC AAGAAAGTTG  
801 GTGAGAGGCC AGCACAGGGA GGGAGGGTGT CTGCTGGAAG CCAGGCTCAG  
851 CGCTCCTGCC TGGACGCATC CCGGCTATGC AGCCCCAGTC CAGGGCAGCA  
901 AGGCAGGCCC CGTCTGCCTC TTCACCCGGA GGCCTCTGCC CGCCCCACTC  
951 ATGCTCAGGG AGAGGGTCTT CTGGCTTTTT CCCCAGGCTC TGGGCAGGCA  
1001 CAGGCTAGGT GCCCCTAACC CAGGCCCTGC ACACAAAGGG GCAGGTGCTG  
1051 GGCTCAGACC TGCCAAGAGC CATATCCGGG AGGACCCTGC CCCTGACCTA  
1101 AGCCCACCCC AAAGGCCAAA CTCTCCACTC CCTCAGCTCG GACACCTTCT  
1151 CTCCTCCCAG ATTCCAGTAA CTCCCAATCT TCTCTCTGCA GAGCCCCAAT  
1201 CTTGTGACAA AACTCACACA TGCCACCGT GCCCAGGTAA GCCAGCCCAG  
1251 GCCTCGCCCT CCAGCTCAAG GCGGGACAGG TGCCCTAGAG TAGCCTGCAT  
1301 CCAGGGACAG GCCCCAGCCG GGTGCTGACA CGTCCACCTC CATCTCTTCC

1351 TCAGCACCTG AACTC<sup>235</sup>CTGG <sup>237</sup>GGACCGTCA GTCTTCCTCT TCCCCCAAA  
 1401 ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG  
 1451 TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG  
 1501 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA  
 1551 CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT  
 1601 GGCTGAATGG CAAG<sup>318</sup>GAGTAC <sup>320</sup>AAGTGC <sup>322</sup>AAG TCTCCAACAA AGCCCTCCCA  
 1651 <sup>331</sup>GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGTGGGA CCCGTGGGGT  
 1701 GCGAGGGCCA CATGGACAGA GGCCGGCTCG GCCCACCTC TGCCCTGAGA  
 1751 GTGACCGCTG TACCAACCTC TGTCCCTACA GGGCAGCCCC GAGAACCACA  
 1801 GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG AACCAGGTCA  
 1851 GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG  
 1901 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT  
 1951 GCTGGACTCC GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA  
 2001 AGAGCAGGTG GCAGCAGGGG AACGTCTTCT CATGCTCCGT GATGCATGAG  
 2051 GCTCTGCACA ACCACTACAC GCAGAAGAGC CTCTCCCTGT CTCCGGGTAA  
 2101 ATGAGTGCGA CGGCCGGCAA GCCCCCGCTC CCCGGGCTCT CGCGGTCGCA  
 2151 CGAGGATGCT TGGCACGTAC CCCCTGTACA TACTTCCCGG GCGCCCAGCA  
 2201 TGGAAATAAA GCACCCAGCG CTGCCCTGGG CCCCTGCGAG ACTGTGATGG  
 2251 TTCTTTCCAC GGGTCAGGCC GAGTCTGAGG CCTGAGTGGC ATGAGGGAGG  
 2301 CAGAGCGGGT CCCACTGTCC CCACACTGGC CCAGGCTGTG CAGGTGTGCC  
 2351 TGGGCCCCCT AGGGTGGGGC TCAGCCAGGG GCTGCCCTCG GCAGGGTGGG  
 2401 GGATTTGCCA GCGTGGCCCT CCCTCCAGCA GCACCTGCCC TGGGCTGGGC  
 2451 CACGGGAAGC CTAGGAGACC CTTGGGGACA GACACACAGC CCCTGCCTCT  
 2501 GTAGGAGACT GTCCTGTTCT GTGAGCGCCC CTGTCCTCCC GACCTCCATG  
 2551 CCCACTCGGG GGCATGCCTA GTCCATGTGC GTAGGGACAG GCCCTCCCTC  
 2601 ACCCATCTAC CCCACGGCA CTAACCCCTG GCTGCCCTGC CCAGCCTCGC  
 2651 ACCCGCATGG GGACACAACC GACTCCGGGG ACATGCACTC TCGGGCCCTG  
 2701 TGGAGGGACT GGTGCAGATG CCCACACACA CACTCAGCCC AGACCCGTTC  
 2751 AACAAACCCC GCACTGAGGT TGGCCGGCCA CACGGCCACC ACACACACAC  
 2801 GTGCACGCCT CACACACGGA GCCTCACCCG GGCGAACTGC ACAGCACCCA

FIGURE 18B

29156

2851 GACCAGAGCA AGG CCTCGC ACACGTGAAC ACTCCTCGGA CACAGGCCCC  
2901 CACGAGCCCC ACGCGGCACC TCAAGGCCCA CGAGCCTCTC GGCAGCTTCT  
2951 CCACATGCTG ACCTGCTCAG ACAAAACCAG CCCTCCTCTC ACAAGGGTGC  
3001 CCCTGCAGCC GCCACACACA CACAGGGGAT CACACACCAC GTCACGTCCC  
3051 TGGCCCTGGC CCACTTCCCA GTGCCGCCCT TCCCTGCAGG ACGGATCAGC  
3101 CTCGACTGTG CCTTCTAGTT GCCAGCCATC TGTGTGTTGC CCCTCCCCCG  
3151 TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA  
3201 AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT CTATTCTGGG  
3251 GGGTGGGGTG GGGCAGGACA GCAAGGGGGA GGATTGGGAA GACAATAGCA  
3301 GGCATGCTGG GGATGCGGTG GGCTCTATGG CTTCTGAGGC GGAAAGAACC  
3351 AGCTGGGGCT CTAGGGGGTA TCCCCACGCG CCCTGTAGCG GCGCATTAAAG  
3401 CGCGGCGGGT GTGGTGTTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG  
3451 CCCTAGCGCC CGCTCCTTTC GCTTCTTCC CTTCTTTCT CGCCACGTTC  
3501 GCCGGGCCTC TCAAAAAGG GAAAAAAGC ATGCATCTCA ATTAGTCAGC  
3551 AACCATAGTC CCGCCCCTAA CTCCGCCCAT CCCGCCCTA ACTCCGCCCA  
3601 GTTCCGCCCA TTCTCCGCC CATGGCTGAC TAATTTTTTT TATTTATGCA  
3651 GAGGCCGAGG CCGCCTCGGC CTCTGAGCTA TTCCAGAAGT AGTGAGGAGG  
3701 CTTTTTTGGA GGCCTAGGCT TTTGCAAAA GCTTGGACAG CTCAGGGCTG  
3751 CGATTTCGCG CCAAACCTGA CGGCAATCCT AGCGTGAAGG CTGGTAGGAT  
3801 TTTATCCCCG CTGCCATCAT GGTTGACCA TTGAACTGCA TCGTCGCCGT  
3851 GTCCCAAAAT ATGGGGATTG GCAAGAACGG AGACCTACCC TGGCCTCCGC  
3901 TCAGGAACGA GTTCAAGTAC TTCCAAAGAA TGACCACAAC CTCTTCAGTG  
3951 GAAGGTAAAC AGAATCTGGT GATTATGGGT AGGAAAACCT GGTTCTCCAT  
4001 TCCTGAGAAG AATCGACCTT TAAAGGACAG AATTAATATA GTTCTCAGTA  
4051 GAGAACTCAA AGAACCACCA CGAGGAGCTC ATTTTCTTGC CAAAAGTTTG  
4101 GATGATGCCT TAAGACTTAT TGAACAACCG GAATTGGCAA GTAAAGTAGA  
4151 CATGGTTTGG ATAGTCGGAG GCAGTTCTGT TTACCAGGAA GCCATGAATC  
4201 AACCAGGCCA CCTTAGACTC TTTGTGACAA GGATCATGCA GGAATTTGAA  
4251 AGTGACACGT TTTTCCCAGA AATTGATTG GGGAAATATA AACTTCTCCC  
4301 AGAATACCCA GCGTCTCTCT CTGAGGTCCA GGAGGAAAAA GGCATCAAGT



4351 ATAAGTTTGA AGTCTACGAG AAGAAAGACT AACAGGAAGA TGCTTTCAAG  
4401 TTCTCTGCTC CCCTCCTAAA GCTATGCATT TTTATAAGAC CATGGGACTT  
4451 TTGCTGGCTT TAGATCTCTT TGTGAAGGAA CCTTACTTCT GTGGTGTGAC  
4501 ATAATTGGAC AAACCTACCTA CAGAGATTTA AAGCTCTAAG GTAAATATAA  
4551 AATTTTAAAG TGTATAATGT GTTAAACTAC TGATTCTAAT TGTTTGTGTA  
4601 TTTTAGATTC CAACCTATGG AACTGATGAA TGGGAGCAGT GGTGGAATGC  
4651 CTTTAATGAG GAAAACCTGT TTTGCTCAGA AGAAATGCCA TCTAGTGATG  
4701 ATGAGGCTAC TGCTGACTCT CAACATTCTA CTCCTCCAAA AAAGAAGAGA  
4751 AAGGTAGAAG ACCCCAAGGA CTTTCCTTCA GAATTGCTAA GTTTTTTGAG  
4801 TCATGCTGTG TTTAGTAATA GAACTCTTGC TTGCTTTGCT ATTTACACCA  
4851 CAAAGGAAAA AGCTGCACTG CTATACAAGA AAATTATGGA AAAATATTCT  
4901 GTAACCTTTA TAAGTAGGCA TAACAGTTAT AATCATAACA TACTGTTTTT  
4951 TCTTACTCCA CACAGGCATA GAGTGTCTGC TATTAATAAC TATGCTCAAA  
5001 AATTGTGTAC CTTTAGCTTT TTAATTTGTA AAGGGGTAA TAAGGAATAT  
5051 TTGATGTATA GTGCCTTGAC TAGAGATCAT AATCAGCCAT ACCACATTG  
5101 TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG  
5151 AAACATAAAA TGAATGCAAT TGTTGTTGTT AACTTGTTTA TTGCAGCTTA  
5201 TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTTACAA AATAAAGCAT  
5251 TTTTTTCACT GCATTCTAGT TGTGGTTTGT CCAAACATCAT CAATGTATCT  
5301 TATCATGTCT GGATCGGCTG GATGATCCTC CAGCGCGGGG ATCTCATGCT  
5351 GGAGTTCTTC GCCCACCCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA  
5401 AATAAAGCAA TAGCATCACA AATTTACAAA ATAAAGCATT TTTTCACTG  
5451 CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG  
5501 TATACCGTCG ACCTCTAGCT AGAGCTTGGC GTAATCATGG TCATAGCTGT  
5551 TTCCTGTGTG AAATTGTTAT CCGCTCACA TTCCACACAA CATACGAGCC  
5601 GGAAGCATAA AGTGTAAGC CTGGGGTGCC TAATGAGTGA GCTAACTCAC  
5651 ATTAATTGCG TTGCGCTCAC TGCCCGCTTT CCAGTCGGGA AACCTGTCTG  
5701 GCCAGCTGCA TTAATGAATC GGCCAACGCG CGGGGAGAGG CGGTTTGCCT  
5751 ATTGGGCGCT CTTCCGCTTC CTCGCTCACT GACTCGCTGC GCTCGGTCTG  
5801 TCGGCTGCCG CGAGCGGTAT CAGCTCACTC AAAGGCGGTA ATACGGTTAT

5351 CCACAGAATC AGGGGATAAC GCAGGAAAGA ACATGTGAGC AAAAGGCCAG  
5901 CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG  
5951 GCTCCGCCCC CCTGACGAGC ATCACA AAAA TCGACGCTCA AGTCAGAGGT  
6001 GGC GAAACCC GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC  
6051 TCCCTCGTGC GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC  
6101 CGCCTTTCTC CCTTCGGGAA GCGTGGCGCT TTCTCAATGC TCACGCTGTA  
6151 GGTATCTCAG TTCGGTGTAG GTCGTTGCT CCAAGCTGGG CTGTGTGCAC  
6201 GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA ACTATCGTCT  
6251 TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG  
6301 GTAACAGGAT TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG  
6351 AAGTGGTGGC CTAAC TACGG CTACACTAGA AGGACAGTAT TTGGTATCTG  
6401 CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT  
6451 CCGGCAAACA AACCACCGCT GG TAGCGGTG GTTTTTTTGT TTGCAAGCAG  
6501 CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC  
6551 TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGT TAA GGGATTTTGG  
6601 TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATTAAAAA  
6651 TGAAGTTTTA AATCAATCTA AAGTATATAT GAGTAAACTT GGTCTGACAG  
6701 TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC TGTCTATTTT  
6751 GTTCATCCAT AGTTGCCTGA CTCCCCGTCG TG TAGATAAC TACGATACGG  
6801 GAGGGCTTAC CATCTGGCCC CAGTGCTGCA ATGATACCGC GAGACCCACG  
6851 CTCACCGGCT CCAGATTTAT CAGCAATAAA CCAGCCAGCC GGAAGGGCCG  
6901 AGCGCAGAAG TGGTCCTGCA ACTTTATCCG CCTCCATCCA GTCTATTAAT  
6951 TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG CCAGTTAATA GTTTGCGCAA  
7001 CGTTGTTGCC ATTGCTACAG GCATCGTGGT GTCACGCTCG TCGTTTGGA  
7051 TGGCTTCATT CAGCTCCGGT TCCCAACGAT CAAGGCGAGT TACATGATCC  
7101 CCCATGTTGT GCAAAAAAGC GGTTAGCTCC TTCGGTCCTC CGATCGTTGT  
7151 CAGAAGTAAG TTGGCCGCAG TGTTATCACT CATGGTTATG GCAGCACTGC  
7201 ATAATTCTCT TACTGTCATG CCATCCGTAA GATGCTTTTC TGTGACTGGT  
7251 GAGTACTCAA CCAAGTCATT CTGAGAATAG TGTATGCGGC GACCGAGTTG  
7301 CTCTTGCCCG GCGTCAATAC GGGATAATAC CGCGCCACAT AGCAGAACTT

FIGURE 18E

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7351 TAAAAGTGCT CATCATTGGA AAACGTTCTT CGGGGCGAAA ACTCTCAAGG  
7401 ATCTTACCGC TGTGAGATC CAGTTCGATG TAACCCACTC GTGCACCCAA  
7451 CTGATCTTCA GCATCTTTTA CTTTCACCAG CGTTTCTGGG TGAGCAAAAA  
7501 CAGGAAGGCA AAATGCCGCA AAAAAGGGAA TAAGGGCGAC ACGGAAATGT  
7551 TGAATACTCA TACTCTTCCT TTTTCAATAT TATTGAAGCA TTTATCAGGG  
7601 TTATTGTCTC ATGAGCGGAT ACATATTTGA ATGTATTTAG AAAAATAAAC  
7651 AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCACC TGACGTCGAC  
7701 GGATCGGGAG ATCTGCTAGG TGACCTGAGG CGCGCCGGCT TCGAATAGCC  
7751 AGAGTAACCT TTTTTTTTAA TTTTATTTTA TTTTATTTT GAGATGGAGT  
7801 TTGGCGCCGA TCTCCCGATC CCCTATGGTC GACTCTCAGT ACAATCTGCT  
7851 CTGATGCCGC ATAGTTAAGC CAGTATCTGC TCCCTGCTTG TGTGTTGGAG  
7901 GTCGCTGAGT AGTGCGCGAG CAAAATTTAA GCTACAACAA GGCAAGGCTT  
7951 GACCGACAAT TGCATGAAGA ATCTGCTTAG GGTAGGCGT TTTGCGCTGC  
8001 TTCGCGATGT ACGGGCCAGA TATACGCGTT GACATTGATT ATTGACTAGT  
8051 TATTAATAGT AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA  
8101 GTTCCGCGTT ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA  
8151 ACGACCCCCG CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG  
8201 CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGACTATT TACGGTAAAC  
8251 TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA  
8301 TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG  
8351 ACCTTATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC  
8401 TATTACCATG GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC  
8451 GGT TTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG  
8501 AGTTTGTTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA  
8551 CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT  
8601 ATATAAGCAG AGCTCTCTGG CTAAGTAGAG AACCCTAGC TTACTGGCTT  
8651 ATCGAAATTA ATACGACTCA CTATAGGGAG ACCCAAGCTT

FIGURE 18F

FIGURE 19 A

pD17-hG1b

10 20 30 40 50 60  
GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC GGTCAATCGA  
CCATGGTTAA ATTAACTAT AGAGGAATCC AGAGCTCAGA GATCTATTGG CCAGTTAGCT

70 80 90 100 110 120  
TTTGAATICT TGCGGCCGCT TGCTAGCACCC AAGGGCCCAT CCGTCTTCCC CCTGGCACCC  
AACCCTAAGA ACGCCGGCGA ACGATCGTGG TTCCCGGGTA GCCAGAAGGG GGACCGTGGG

130 140 150 160 170 180  
TCC'TCCAAGA GCACCTCTGG GGGCACAGCG GCCCTGGGCT GCCTGGTCAA GGACTACTTC  
AGGAGTICT CGTGGAGACC CCGTGTCTGC CCGGACCCGA CCGACCAATT CCTGATGAAG

190 200 210 220 230 240  
CCCGAACCGG TGACGGTCTC GTGGAACCTA GCGGCCCTGA CCAGCGGCGT GCACACCTTC  
GGCTTGGCC ACTGCCACAG CACCTTGAGT CCGCGGGACT GGTGCGCGCA CGTGTGGAAG

250 260 270 280 290 300  
CCGGCTCTCC TACAGTCTTC AGGACTCTAC TCCCTCAGCA GCGTGGTCAC CGTGGCCCTCC  
GGCCGACAGG ATGTCAAGGAG TCCTGAGATG AGGGAGTCTG CGCACCAATG GCACGGGAGG

310 320 330 340 350 360  
AGCAGCTTGG GCACCCAGAC CTACATCTGC AACGTGAATC ACAAGCCAG CAACACCAAG  
TCGTGGAACC CGTGGGTCTG GATGTAGACG TTGCACCTAG TGTTCGGGTC GTTGTGGTTC

370 380 390 400 410 420  
GTGGACAAGA AAGTTGGTGA GAGGCCAGCA CAGGGAGGGA GGGTGTCTGC TGAAGCCAG  
CACCTGTCT 'TCAACCACT CTCCGGTCTG GTCCCTCCCT CCCACAGACG ACCTTCGGTC

430 440 450 460 470 480  
GCTCAGCGT CCTGCCCTGGA CGCATCCCGG CTATGACAGCC CCAGTCCAGG GCAGCAAGGC  
CGAGTCGCGA GGACGGACCT GCGTAGGGCC GATACGTCCG GGTACAGTCC CGTCTGTCGG

490 500 510 520 530 540  
AGGCCCCGTC TGCTCTTTCA CCGGAGGGCC TCTGCCCCGCC CCACTCATGC TCAGGGAGAG  
TCCGGGSCAG ACGGAGAAGT GGGCCTCCCG AGACGGGCGG GGTGAGTACG AGTCCCTCTC

550 560 570 580 590 600  
GGTCTTCTGG CTTTTTCCCC AGGCTCTGGG CAGGCACAGG CTAGGTGCC CTAACCCAGG  
CCACAGACCC GAAAAGGGG TCCGAGACCC GTCCGTGTC GATCCACGGG GATGGGTCC

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FIGURE 19B

## pD17-hG1b

610 620 630 640 650 660  
 CCCTGCACAC AAAGGGGAG GTGCTGGCT CAGACCTGCC AAGAGCCATA TCCGGGAGGA  
 GGGACCTGTG TTTCCTCCGTC CACGACCCGA GTCTGGACGG TTCTCGGTAT AGGCCCTCCT  
  
 670 680 690 700 710 720  
 CCTGCCCCCT GACCTAAGCC CACCCCAAG GCCAACTCT CCACCTCCCTC AGCTCGGACA  
 GGGACGGGA CTGGATTCCG GTGGGGTTTC CGTTTGAGA GGTGAGGGAG TCGAGCCTGT  
  
 730 740 750 760 770 780  
 CCTTCTCTTC TCCAGATTC CAGTAACCTC CAATCTTCTC TCTGCAGAGC CCAAATCTTG  
 GGAAGAGAGG AGGTCTAAG GTCAATTGAG GTTAGAAGAG AGACGTCTCG GGTTTAGAAC  
  
 790 800 810 820 830 840  
 TGACAAACT CACACATGCC CACCGTGCC AGGTAAGCCA GCCCAGGCTT CGCCCTCCAG  
 ACTGTATTGA GTGTGTACGG GTGGCACGG TCCATTCCGT CGGTCCTCGA GCGGGAGGTC  
  
 850 860 870 880 890 900  
 CTCAGAGCG GACAGGTGCC CTAGAGTAG CTGACATCCAG GGACAGGCC CAGCCGGGTG  
 GAGTTCCGCC CTGTCCACGG GATCTCATG GACGTAGGTC CCTGTCCGG GTCCGGCCAC  
  
 910 920 930 940 950 960  
 CTGACACGTC CAGCTCCATC TCTTCTCTCAG CACCTGAACT CTCTGGGGA CCGTCAGTCT  
 GACTGTGCAG GTGGAGGTAG AGAAGGAGTC GTGGACTTGA GACCTCCCTT GGCAGTCAGA  
  
 970 980 990 1000 1010 1020  
 TCTCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC CCGACCCCT GAGGTACAT  
 AGGAGNAGG GGGTTTGGG TTCTCTGTGG GTTACATAGAG GCCCTGGGA CTCCAGTGA  
  
 1030 1040 1050 1060 1070 1080  
 GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG TACGTGGACG  
 CGCACCCACA CCTGCACTCG GTGCTTCTGG GACTCCAGTT CAAGTTGACC ATGCACCTGC  
  
 1090 1100 1110 1120 1130 1140  
 CCGTGGAGGT GCATATGCC AAGACAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC  
 CGCACCTTCA CGTATTTACGG TTCTGTCTCG GCGCCCTTCTT CGTCATGTTG TCGTGCATGG  
  
 1150 1160 1170 1180 1190 1200  
 GTGTGGTTCAG CGTCCTCACC GTCTGTGACC AGGACTGGCT GAATGGCAAG GAGTACAGT  
 CACACCTATC GCAGGAGTGG CAGGACGTGG TCCTGACCCA CTTACCGTTC CTCATCTCA

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FIGURE 19C

pD17-hG1b

322	1210	1220	1230	1240	1250	1260
CAAGGCTC	CAACAAAGCC	CTCCAGCC	CCATCGAGAA	AACCATCTCC	AAAGCCAAAG	
CTTCCAGAG	GTGTGTTCCG	GAGGTCGG	GGTAGCTCTT	TTGGTAGAGG	TTTCGGTTTC	
1270	1280	1290	1300	1310	1320	
GTGGGACCCG	TGGGGTCCGA	GGGCCACATG	GACAGAGGCC	GGCTCGGCC	ACCTCTTGCC	
CACCTGGGC	ACCCACGCT	CCCGGTGTAC	CTGTCTCCGG	CCGAGCCGG	TGGGAGACGG	
1330	1340	1350	1360	1370	1380	
CTGAGAGTGA	CCGCTGTACC	AACCTCTGTC	CCTACAGGGC	AGCCCCGAGA	ACCACAGGTG	
GACTCTCACT	GGCGACATGG	TTGGAGACAG	GGATGTCCCG	TCGGGGCTCT	TGGTGTCCAC	
1390	1400	1410	1420	1430	1440	
TACACCTTGC	CCCCATCCCG	GGATGAGCTG	ACCAAGAACC	AGGTCAGCCT	GACCTGCCTG	
ATGTGGGACG	GGGGTAGGGC	CCTACTCGAC	TGGTCTTGG	TCCAGTCGGA	CTGGACGGAC	
1450	1460	1470	1480	1490	1500	
GTCAAGGCT	TCTATCCAG	CGACATCCGC	GTGGAGTGG	AGAGCAATGG	GCAGCCGGAG	
CAGTTTCCGA	AGATAGGGTC	GCTGTAGCGG	CACCTCACCC	TCTCGTTACC	CGTCGGCCTC	
1510	1520	1530	1540	1550	1560	
AACAACCTACA	AGACCACGCC	TCCCGTGTCTG	GACTCCGACG	GCTCCTTCTT	CCTCTACAGC	
TTGTGTGATGT	TCTGGTCCGG	AGGGCACGAC	CTGAGGCTGC	CGAGGAAGAA	GGAGATGTCTG	
1570	1580	1590	1600	1610	1620	
AAGCTCACCG	TGGACAAGAG	CAGGTGGCAG	CAGGGGAACG	TCTTCTCATG	CTCCGTGATG	
TTCCGAGTGGC	ACCTGTCTC	GTCCACCGTC	GTCCCCCTTC	AGAAGAGTAC	GAGGCACTAC	
1630	1640	1650	1660	1670	1680	
CATCAGGCTC	TGCACAACCA	CTACACGAG	AAGAGCCCT	CCCCTGTCTCC	GGGTAAATGA	
GTACTCCGAG	ACGTGTTGGT	GATGTGGCTC	TTCTCGGAGA	GGGACAGAGG	CCCATTTACT	
1690	1700	1710	1720	1730	1740	
GTGCGACGSC	CGGCAAGCCC	CCGCTCCCG	GGCTCTCGCG	GTCCGACGAG	GATGCTTGGC	
CACGCTGCGG	GCCGTTCGGG	GGCGAGGSGC	CCGAGAGCGC	CAGCGTGTCT	CTACGNAACCG	
1750	1760	1770	1780	1790	1800	
ACGTACCCCC	TGTACATACT	TCCCGGGCGC	CCAGCATGGA	AATTAAGCAC	CCAGCGCTGC	
TGCATGGGG	ACAATATGA	AGGGCCCGCG	GGTCGTACCT	TTATTTCTGTG	GGTCGGGACG	

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FIGURE 19D

pD17-hG1b

1810	1820	1830	1840	1850	1860
CCCTGGC'CCC	TGCGAGACTG	TGATGGTTC	TTCCACGGGT	CAGGCCGAGT	CTGAGGCCCTG
GGACCCGGGG	ACGCTCTGAC	ACTACCAAGA	AAGGTGCCCCA	GTCCGGGTCA	GACTCCGGAC
1870	1880	1890	1900	1910	1920
AGTGGC'ATGA	GGGAGGCAGA	GCGGGTCCCA	CTGTCCCCAC	ACTGGCCACG	GCTGTGCAGG
TCACCC'GACT	CCCTCCGTCT	CGCCACGGGT	GACAGGGGTG	TGACCCGGTC	CGACACGTCC
1930	1940	1950	1960	1970	1980
TG'NCC'TGGG	CCCCCTAGGG	TGGGGCTCAG	CCAGGGGCTG	CCC'TCGGCAG	GGTGGGGGAT
ACACGG'ACC	GGGGGATCCC	ACCCCGAGTC	GGTCCCCCGAC	GGGAGCCGTC	CCACCCCCCTA
1990	2000	2010	2020	2030	2040
TTGCCAGCGT	GGCCCTCCCT	CCAGCAGCAC	CTGCCCTGGG	CTGGGCCACG	GGAAGCCCTTA
AACGGTC'GCA	CCGGGAGGGA	GGTCGTCTG	GACGGGACCC	GACCCGCTGC	CCTTCGGGAT
2050	2060	2070	2080	2090	2100
GGAGCC'CTTG	GGGACAGACA	CACAGCCCT	GCCTCTG'FAG	GAGACTGTCC	TGTTCTGTGA
CC'TCGGGAC	CCCTGTCTGT	G'GTCTGGGGA	CGGAGACATC	CTCTGACAGG	ACAAGACACT
2110	2120	2130	2140	2150	2160
GCGCC'CTGT	CC'TCCCGACC	TCCATGCCCA	CTCGGGGGCA	TGC'TGGGAT	GCGGTGGGCT
CGCGGG'GACA	GGAGGGCTGG	AGGTACGGGT	GAGCCCCCGT	ACGACCCCTA	CGCCACCCGA
2170	2180	2190	2200	2210	2220
C'FA'IGG'ITC	TGAGGGCGAA	AGAACCACT	GGGGCTCTAG	GGGGTATCCC	CACGGGCCCT
GATACCG'GAG	ACTCCGCC'IT	TC'TTGGTCGA	CCCCGAGATC	CCCCATAGGG	GTGCGGGGA
2230	2240	2250	2260	2270	2280
GTAGCG'CGC	ATTAAGCGCG	GCGGGTGTGG	TGGTTACGCG	CAGCGTGACC	GCTACACTTG
CATCGCC'CGG	'TAATTGCGC	CGCCCAAC	ACCAATGCGC	GTCCGACTGG	CGATGTGAAC
2290	2300	2310	2320	2330	2340
CCAGCC'CCCT	AGCGCCCGCT	CC'TTTCGCTT	TCTTCCCTTC	C'TTTCGCCC	ACGTTCCGCC
GGTCGCG'GGA	TGCGGGCGGA	GGAAAGCGAA	AGAAGGGGAG	GAAAGAGCGG	TGCAAGCGGC
2350	2360	2370	2380	2390	2400
GCTTTC'CCCG	TCAAGCTCTA	AATCGGGGCA	TCCCTTTAGG	GTTCGATTTT	AGTGC'TTTAC
CGAAAG'GSGC	AGTTCGAGAT	TTAGCCCCCT	AGGGAATCC	CAAGGCTAAA	TCACGNAATG

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FIGURE 19E

pD17-hG1b									
2410	2420	2430	2440	2450	2460				
GGCACCTCGA	CCCCAAAAA	CTTGATTAGG	GTGATGCTTC	ACGTAGTGG	CCATCGCCCT				
CCGTGGAGCT	GGGGTTTTT	GAACTAATCC	CACTACCAAG	TGCATCACCC	GGTAGCGGA				
2470	2480	2490	2500	2510	2520				
GATAGACGGT	TTTTCGCCCT	TTGACGTTGG	AGTCCACGTT	CTTTAATAGT	GGACTCTTGT				
CTATCTGCCA	AAAAGCGGA	AAC TGCAACC	TCAGGTGCAA	GAAATTATCA	CCTGAGAAC				
2530	2540	2550	2560	2570	2580				
TCCAAAC'NGG	AACAACACTC	AACCCATATCT	CGGTCTATTC	TTTTGATTTA	TAAGGGATTT				
AGGTTTGACC	TTGTTTGAG	TTGGGATAGA	GCCAGATAAG	AAACTAAAT	ATTCCCTAAA				
2590	2600	2610	2620	2630	2640				
TGGGGATTTC	GGCCTATTGG	TTAAAAAATG	AGCTGATTTA	ACAAAAATTT	AACGGGAATTT				
ACCCCTAAAG	CCGGATAACC	AATTTTTTAC	TCGACTAAAT	TGTTTTTAAA	TTGCGCTTAA				
2650	2660	2670	2680	2690	2700				
AATCTGTGG	AATGTGTCTC	AGTTAGGTG	TGGAAGTCC	CCAGGCTCCC	CAGGCAGGCA				
TTAAGACACC	TTACACACAG	TCAATCCAC	ACCTTTCAGG	GGTCCGAGGG	GTCCGTCCGT				
2710	2720	2730	2740	2750	2760				
GAAGTATGCA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	CTAACTCCGC				
CTTCATACGT	TTTCGTACGA	GAGTTAATCA	GTGTTTGTA	TCAGGGCGGG	GATTGAGGCG				
2770	2780	2790	2800	2810	2820				
CCATCCCGCC	CCTAACTCCG	CCCAGTTCCG	CCCATTCCTC	GCCCCATGGC	TGACTAAATTT				
GGTAGGGCGG	GGATTGAGGC	GGTCAAGGC	GGTAAGAGG	CGGGGTACCG	ACTGATTAAA				
2830	2840	2850	2860	2870	2880				
TTTTTATTTA	TGCAGAGGCC	GAGGCGCCCT	CGGCCCTCTGA	GCTATTCCAG	AAGTAGTGAG				
AAAAATAAAT	ACGTCCTCCG	CTCCGGCGGA	GCCGGAGACT	CGATAAGGTC	TTTCATCCTC				
2890	2900	2910	2920	2930	2940				
GAGGCTTTT	TGGAGGCCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	GCTGCGATTT				
CTCCGAAAAA	ACCTCCGGAT	CCGAAAAACGT	TTTTTCGAAC	TGTCGAGTCC	CGACGCTAAA				
2950	2960	2970	2980	2990	3000				
CGGCGCAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	CCCGCTGCCA				
GGCGCTTTTC	AACTGCCGT	AGGATCGCAC	TTCCGACCAT	CCAAAAATAG	GGCGACCGT				

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FIGURE 19F

pD17-hG1b

3010	3020	3030	3040	3050	3060
TCATGTTCG	ACCATGAA	TGCATCGT	CCGTGTCCCA	AAATATGGG	ATTGGCAAGA
AGTACCAAG	TGGTAAC	TGACGAGC	GGCACAGGT	TTTATACCC	TAAACCGTCT
3070	3080	3090	3100	3110	3120
ACGGAGACCT	ACCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	AGAATGACCA
TGCCCTCTGA	TGGGACCGGA	GGCGAGTCCT	TGCTCAAGTT	CATGAAGGT	TCCTACTGGT
3130	3140	3150	3160	3170	3180
CAACCTCTTC	AGTGAAGGT	AAACAGATC	TGGTGATAT	GGGTAGGAAA	ACCTGGTTCT
GTGGAGAG	TCACCTTCCA	TTTGTCTTAG	ACCACTAATA	CCCATCCCTT	TGGACCAAGA
3190	3200	3210	3220	3230	3240
CCATTCCTGA	GAAGAACTGA	CCTTTAAAG	ACAGAAATAA	TATAGTTCTC	AGTAGAGAAC
GGTAAGGACT	CTTCTTAGCT	GGAAATTTCC	TGCTCTTAAT	ATATCAAGAG	TCATCTCTTG
3250	3260	3270	3280	3290	3300
TCAAGAACC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG	TTTGGATGAT	GCCTTAAGAC
AGTTTCTTGG	TGGTGTCTCT	CGAGTAAAG	AACGGTTTTC	AAACCTACTA	CGGAATCTG
3310	3320	3330	3340	3350	3360
TTATTAACA	ACCGGAATG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	GGAGGCAGTT
AAATAACTTGT	TGGCCTTAAC	CGTTCAATTC	ATCTGTACCA	AACCTATCAG	CCTCCGTCAA
3370	3380	3390	3400	3410	3420
CTGTTTACCA	GGAAGCCATG	AAATCAACCAG	GCCACCTTAG	ACTCTTTGTG	ACAAGGATCA
GACAAATGGT	CTTTCGGTAC	TTAGTGTGTC	CGGTGGAAATC	TGAGAAACAC	TGTTCTTAGT
3430	3440	3450	3460	3470	3480
TGCAGGAAT	TGAAAGTGAC	ACGTTTCTCC	CAGAAATGA	TTTGGGGAAA	TATAAACTTC
ACGTCTCTAA	ACTTTCAC	TGCANAAAGG	GTCTTTAACT	AAACCCCTTT	ATATTTGAAG
3490	3500	3510	3520	3530	3540
TCCCAGAATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	AAGTATAAGT
AGGGCTTAT	GGGTCCGCAG	GAGAGACTCC	AGGTCCICCT	TTTTCCGCTAG	TTTCATATTC
3550	3560	3570	3580	3590	3600
TTGAGTCTTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	GCTCCCCCTCC
AACTTCAGAT	GCTCTCTCTT	CTGATTTGTC	TTCTACGAAA	GTTCAGAGA	CGAGGGGAGG

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FIGURE 19C

## pD17-hG1b

3610 TAAAGCTATG CATTTTATATA AGACCATGGG ACITTTTGCCTG GCTTTAGATC TCITTTGTGAA 3660  
ATTTCGATAC GTAAAAATAT TCTGGTACCC TGAAAACGAC CGAAATCTAG AGAAACACTT

3670 GGAACCTTAC TTCTGTGGTG TGACATAATT GGACAAACTA CCTACAGAGA TTATAAAGCTC 3720  
CCTTGGATG AAGACACCAC ACTGTATTAA CCTGTTTGAT GGATGCTCTT AAATTTTCGAG

3730 TAAAGTAAAT ATAAAAATTT TAAAGTGATA ATGIGTTAAA CTACTGATTC TAATTCGTTTG 3780  
ATTCCATTTA TATTTTAAAA ATTACATAT TACACAAATTT GATGACTAAG ATTAACAAC

3790 TGTATTTTAG ATTCCAACCT ATGGAACCTGA TGAATGGGAG CAGTGGTGA ATGCCCTTTTAA 3840  
ACATAAAATC TAAGGTGGA TACCTTGACT ACTTACCCTC GTCACCACCT TACGGAAAT

3850 TGAGGAAAC CTGTTTTCCT CAGAAGAAAT GCCATCTAGT GATGATGAGG CTACTGCTGA 3900  
ACTCCTTTTG GACAAAACGA GTCTTCTTTA CGGTAGATCA CTACTACTCC GATGACGACT

3910 CTCCTAACAT TCTACTCTC CAAAAAGAA GAGAAAGGTA GAAGACCCCA AGGACTTTCC 3960  
GAGAGTGTGA AGATGAGGAG GTTTTTCCTT CTCTTTCCAT CTCTGGGGT TCCTGAAAGG

3970 TTTGAGATG CTAAGTTTAT TGAGTCATGC TGCTTTTAGT AATAGAACTC TTGCTTTGCTT 4020  
AAGTCTTAAC GATTCAAAAA ACTCAGTACG ACACAAATCA TTATCTTGAG AACGAACGAA

4030 TGCTATTAC ACCACAAAGG AAAAGCTGC ACTGCTATAC AAGAAAATTA TGGAAAAATA 4080  
ACGATATATG TGGTGTTCCT TTTTTCGACG TGACGATATG TTCTTTTAAT ACCTTTTAT

4090 TTTCTGTAAC TTTATATAGTA GGCATAACAG TTATATATCAT AACATACTGT TTTTCTTAC 4140  
AAGACAT'GG AATATATTCAT CCGTATTGTC AATATTAGTA TTGTATGACA AAAAAGAATG

4150 TCCACACAGG CATAGAGTGT CTGCTATTAA TAACATGCT CAAAAATGT GTACCTTTAG 4200  
AGGTGTCTCC GTATCTCACA GACGATATTT ATTGATACCA GTTTTTRACA CATGAAATC

FIGURE 19H

pD17-hG1b

4210	4220	4230	4240	4250	4260
CTTTTATATT	TGTAAAGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCCT	TGACTAGAGA
GAAAAATTAA	ACATTTCCT	AAATTATCCT	TATAAACTAC	ATATCACGGA	ACTGATCTCT
4270	4280	4290	4300	4310	4320
TCATPAATCAG	CCATACCACA	TTTGTAGAGG	TTTTACTTGC	TTTAAAAAAC	CTCCACACCC
AGTATTAGTC	GGTATGGTGT	AAACATCTCC	AAAATGAACG	AAATTTTGTG	GAGGGTGTGG
4330	4340	4350	4360	4370	4380
TCCCCCTGAA	CCTGAACAT	AAAATGAATG	CAATGTGTGT	TGTTAACTTG	TTTATTGTCAG
AGGGGGACTT	GGACTTTGTA	TTTTACTTAC	GTTAACNACA	ACAATTGAAC	AAATTAACGTC
4390	4400	4410	4420	4430	4440
CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	GCATTTTCTT
GAATATTACC	AATGTTTATT	TCGTTATCGT	AGTGTTTAAA	GTTGTTTATT	CGTAAAAAAA
4450	4460	4470	4480	4490	4500
CACTGCATTC	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	GTCGTGGATCG
GTGACGTAAG	ATCAACACCA	AACAGGTTTG	AGTAGTTTACA	TAGAATAGTA	CAGACCTAGC
4510	4520	4530	4540	4550	4560
GCTGGATGAT	CCCTCCAGCG	GGGATCTCA	TGCTGGAGTT	CTTCGCCAC	CCCAACTTGT
CGACCTACTA	GGAGGTCGCG	CCCTTAGAGT	ACGACCTCAA	GAGCGGGTG	GGGTTGAACA
4570	4580	4590	4600	4610	4620
TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTT	ACAAATAAAG
AATAACGTCG	AATATTACCA	ATGTTTATTT	CGTTATCGTA	GTTTATAAAG	TGTTTATTTT
4630	4640	4650	4660	4670	4680
CAITTTTCTC	ACITGCTTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	TCTTATCATG
GTAATAAAG	TGACGTAAGA	TCAACACCAA	ACAGGTTTGA	GTAGTTACAT	AGAATAGTAC
4690	4700	4710	4720	4730	4740
TCTGTATACC	GTGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTTCTCTG
AGACATATGG	CAGCTGGAGA	TCGATCTCGA	ACCGCATTTAG	TACCAGTATC	GACAAAGGAC
4750	4760	4770	4780	4790	4800
TGTCGAATTG	TTATCCGCTC	ACAAATCCAC	ACAACATACG	AGCCGGAAGC	ATAAAGTCTA
ACACTTTAAAC	AATAGGCGAG	TGTTAAGGTG	TGTTGTTNCC	TGGGCTTTCG	TATTTTCACAT

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FIGURE 191

pD17-hG1b

4810	4820	4830	4840	4850	4860
AAGCC'GGG	TGCCTAATGA	G'GAGCTAAC	TCACATTAA	TGCGTTGCG	TCACTGCCCG
TT'CGGACCCC	ACGGATTACT	CACTCGATTG	AGTGTAAATTA	ACGCAACGCG	AGTGACGGGC
4870	4880	4890	4900	4910	4920
CTTTCCAGTC	GGGAAAC'CTG	TCGTGCCAGC	TGCATTAAATG	AATCGGCCAA	CGCGCGGGGA
GAAAGGTCAG	CCCTTTGGAC	AGCACGGTCG	ACGTAAT'TAC	TTAGCCCGGT	GCGCGCCCCCT
4930	4940	4950	4960	4970	4980
GAGCGCGT'TT	GCGTATTGGG	CGCTCTTTCCG	CTTCCTCGCT	CACTGACTCG	CTGCGGCTCGG
CTCCGCCCAA	CGCATAAACC	CGGAGAAGGC	GAAGGAGCGA	GTGACTGAGC	GACCGAGCC
4990	5000	5010	5020	5030	5040
TCGTTTCGGCT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG
AGCAAGCCGA	CGCCGCTCGC	CATAGTCGAG	TGAGTTTCCG	CCATTATGCC	AATAGGTGTC
5050	5060	5070	5080	5090	5100
AATCAGGGGA	TAACGCAGGA	AAGAACAATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC
TTAGTCCCCCT	ATTGCGTCTT	TTCTTTGTACA	CTCGTTTTC	GGTCGTTTTC	CGGTCTTTCG
5110	5120	5130	5140	5150	5160
GTAATAAGGC	CGCGTTGCTG	GCGTTTTC	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA
CATT'TTTCG	GCGCAACGAC	CGCAAAAAG	TATCCGAGGC	GGGGGACTG	CTCGTAGTGT
5170	5180	5190	5200	5210	5220
AAATTCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT
TTTTAGCTGC	GAGTTCAGTC	TCCACCGCTT	TGGGCTG'ICC	TGATATTTC'T	ATGGTCCGCA
5230	5240	5250	5260	5270	5280
TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC
AAGGGGACC	TTTCGAGGAG	CACGCGAGAG	GACAAGGCTG	GGACGCGGAA	TGGCCTATGG
5290	5300	5310	5320	5330	5340
TGTCCGCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTCTCA	ATGCTCACGC	TGTAGGTATC
ACAGGGGAA	AGAGGGAAGC	CCTTCCGACC	GCGAAAGAGT	TACGAGTGG	ACATCCATAG
5350	5360	5370	5380	5390	5400
TCAGTTCGGT	GTAGGTGTT	CGCTCCAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC
AGTCAAGCCA	CATCCAGCAA	GCGAGGTTCG	ACCCGACACA	CGTGTTCGG	GGGCNAGTGC

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FIGURE 19J

pD17-hG1b

5410	5420	5430	5440	5450	5460
CCGACCGCTG	CGCCTTATCC	GGTAACATATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT
GGCTGGCGAC	GCGGAATAGG	CCATTGATAG	CAGAACTCAG	GTTGGGCCAT	TCTGTGCTGA
5470	5480	5490	5500	5510	5520
TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG
ATAGCGGTGA	CCGTCTGTCG	TGACCATATGT	CCTAATCTGTC	TGCTTCATTA	CATCCGCCAC
5530	5540	5550	5560	5570	5580
CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTGTGTA
GATGTCACAA	GAACCTCACC	ACCGGATTGA	TGCCGATGTG	ATCTTCCCTGT	CATAAACCAT
5590	5600	5610	5620	5630	5640
TCCTGCGTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA
AGACCCGAGA	CGACTTCGGT	CAATGGAAAC	CTTTTCTCTCA	ACCATCGAGA	ACTAGGCCGT
5650	5660	5670	5680	5690	5700
AACAACCCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA
TTGTTTGGTG	GCGACCATCG	CCACCACAAA	AACAAACGTT	CGTCGTCATA	TGCGCGTCTT
5710	5720	5730	5740	5750	5760
AAAAAGGATC	TCAAGAAGAT	CTTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAAACG
TTTTTCTCTAG	AGTCTCTCTA	GGAAACTAGA	AAAGATGCCC	CAGACTGCGA	GTACACCTTGC
5770	5780	5790	5800	5810	5820
AAAACTCACG	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	ACCTAGATCC
TTTTTGACTGC	AATTCCCTAA	AACCACTACT	CTAATAGTTT	TTCCCTAGAAG	TGGATCTAGG
5830	5840	5850	5860	5870	5880
TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAAGTAT	ATATGAGTAA	ACTTGGTCTG
AAAATTTAAAT	TTTTACTTCA	AAATTTAGTT	AGATTTTCATA	TATACTCATTT	TGAACCCAGAC
5890	5900	5910	5920	5930	5940
ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTTCAT
TGTCNAATGT	TACGAATTAG	TCACTCCGTG	GATAGAGTCTG	CTAGACAGAT	AAAGCAAGTA
5950	5960	5970	5980	5990	6000
CCATAGTTGC	CTGACTCCCC	GTCTGTGTAGA	TAACTACGAT	ACGGGAGGSC	TTACCATCTG
GGTATCAACC	GACTGAGGGG	CAGCACATCT	ATTGATGCTTA	TGCCCCCTCCG	AATGGTAGAC

FIGURE 19K

## pD17-hG1b

6010 G C C C C A G T G C T G C A A T G A T A C C G G A G A C C C A C G T C T G G G G G T C A C A C T A C T A T G G C G T C C A G A T T T A T C A G C A A 6060  
6070 T A A A C C A G C C A G C C G G A A G G G C C G A G C G C A G A G T G G T C C T G C A A C T T T A T C C G C T C C A 6120  
6130 T C C A G T C T A T T A A T T G T T G C C G G A A G C T A G A C T A A G C T T C A G T T A A T A G C G G A G G T 6180  
6190 G C A A C G T T G T T G C C A T T G C T A C A G G C A T C G T C C G T A G T C G C C A G T T A T C G C C A G T T A A T A G T T T G C 6240  
6250 C A T T C A G C T C C G T T C C C A A C G A T C A A G G C G A T T A C A T G A T C C C C C A T G A T C C C A A A T T G T G C A A A 6300  
6310 G T A A G T C G A G G C C A A G G T T C C G C T A G T T C C G C T C A A T G T A C T A G G G G T A C A A C A C G T T T T 6360  
6370 A A G C G G T T A G C T C C T T C G G T C C T C C G A T C G T T G T C A G A A G T A A G T T G G C C G C A G T G T T A T 6420  
T T C G C C A T C G A G A C A C T G A C C A C T C A T G A C T A A T T C T C T T A C T G T G A G A A T G A C A G A T G C T 6480  
6490 G T T G C T C T T G C C C G G C G T C A A T A C G G G A T A T A C C G C G C C A C A T A G C A G A A C T T T A A A A G 6540  
6550 T G C T C A T C A T T G A A C A C G T T C T T C G G G C G A A A A C T C T C A A G A G C C C C G C T T T T G A C A G 6600  
A C G A G T A C T A A C C T T T T G C A A G A A G C C C C G C T T T T G A C A G T T C C T A G A A T G C C G A C A A C T

FIGURE 19L

## pD17-hG1b

6610 GATCCAGTTC 6620 GATGTAACCC 6630 ACTCGTGCAC 6640 CCAACTGATC 6650 TTCAGCATCT 6660 TTTACTTTTCA  
CTAGGTC AAG CTACATTTGGG TGAGCACGTG GGTGACTAG AAGTCGTAGA AAATGAAAGT  
6670 CCAGCGTTTC 6680 TGGGTGAGCA 6690 AAAACAGGAA 6700 GGCAAAATGC 6710 CGCAAAAAGG 6720 GGAATAAGGG  
GGTCGCAAG ACCCACCTCGT TTTTGTCTT CCGTTTACG GCGTTTTTC CCTTATTCCC  
6730 CGACACGGAA 6740 ATGTTGAATA 6750 CTCATACTCT 6760 TCCTTTTTCA 6770 ATATTATGA 6780 AGCATTTATC  
GCTGTGCCTT TACAACCTTAT GAGTATGAGA AGGAAAAAGT TATAATAAAT TCGTAAATAG  
6790 AGGGTTATTG 6800 TCTCATGAGC 6810 GGATACATAT 6820 TTGAATGTAT 6830 TTAGAAAAAT 6840 AAACAAATAG  
TCCCAATAAC AGAGTACTCG CCTATGTATA AACTTACATA AATCTTTTTA TTTGTTTTATC  
6850 GGGTTCCCG 6860 CACATTTCCC 6870 CGAAAGTGC 6880 CACCTGACGT 6890 CGACGGATCG 6900 GGAGATCTGC  
CCCAAGGCG GTGTAAGGG GCTTTTCAG GTGGACTGCA GCTGCCTAGC CCTCTAGACG  
6910 TAGGTGACCT 6920 GAGGCGCGCC 6930 GGCTTCGAAT 6940 AGCCAGAGTA 6950 ACCTTTTTTT 6960 TTAATTTTTAT  
ATCCACTGGA CTCCGCGCGG CCGAAGCTTA TCGGTCTCAT TGGAAAAAAA AATTAAAAATA  
6970 TTTTATTTTAT 6980 TTTTGAGATG 6990 GAGTTTGGCG 7000 CCGATCTCCC 7010 GATCCCCCTAT 7020 GGTGACTCT  
AAATAAAATA AAAACTCTAC CTCAAACCCG CCGTATGATA TTCCGGTATA GACGAGGGAC GAACACACAA  
7030 CAGTACAATC 7040 TGCTCTGATG 7050 CCGCATAGTT 7060 AAGCAGTAT 7070 CTGCTCCCCTG 7080 CTTGTGTGTT  
GTCTATGTTAG ACAGACTAC ACAGACTAC GCGTATCAA TTCCGGTATA GACGAGGGAC GAACACACAA  
7090 GGAGTTCGCT 7100 GAGTAGTGGC 7110 CGAGCAAAAT 7120 TTAAGCTACA 7130 ACAAGGCAAG 7140 GCTTGACCGA  
CCTCCAGCGA CTCATCACCG GCTCGTTTTA AATTCGATGT TGTTCGGTTC CGAAGTGGCT  
7150 CAATTGCATG 7160 AAGAACTCG 7170 TTAGGGTTAG 7180 GCGTTTTGCG 7190 CTGCTTCGCG 7200 ATGTACGGGC  
GTTAACGTAC TCTTTAGAC AATCCCAATC CGCAAAACCG GACGAAGCGC TACATGCCCG

FIGURE 19M

pD17-hG1b									
7210	7220	7230	7240	7250	7260				
CAGATATACG	CGTTGACATT	GATTAATGAC	TAGTTATTA	TAGTAATCAA	TTACGGGGTC				
GTCTATATGC	GCAACTGTAA	CTAATAACTG	ATCAATAATT	A1CATTAGTT	AATGCCCCAG				
7270	7280	7290	7300	7310	7320				
ATTAGTTTCAT	AGCCCATATA	TGGAGTTCCG	CGTTACATAA	CTTACGGTAA	ATGGCCCGCC				
TAATCAACTA	TCGGGTATAT	ACCTCAAGGC	GCAATGTATT	GAATGCCATT	TACCGGGCGG				
7330	7340	7350	7360	7370	7380				
TGGCTGACCG	CCCAACGACC	CCCGCCCAT	GACGTCAATA	ATGACGTATG	T1CCCATAGT				
ACCGACTTGGC	GGGTGCTGG	GGCGGGTAA	CTGCAGTTAT	TACTGCATAC	AAGGGTATCA				
7390	7400	7410	7420	7430	7440				
AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGGTGGAC	TATTTACGGT	AAACTGCCCA				
T1TGCSTTAT	CCCTGAAGG	TNACTGCAGT	TACCCACCTG	ATAAATGCCA	TTTGACGGGT				
7450	7460	7470	7480	7490	7500				
CTTGGCAGTA	CATCAAGTGT	ATCATATGCC	AAGTACGCC	CCTATTGACG	TCAATGACGG				
GAACCGTCAT	GTAGTTCACA	TAGTATACGG	TTCATGCGGG	GGATNACTGC	AGTTACTGCC				
7510	7520	7530	7540	7550	7560				
TAAATGGCCC	GCCTGGCATT	ATGCCCACTA	CATGACCTTA	TGGGACTTTTC	CTACTTGGCA				
ATTTTACCGG	CGGACCGTAA	TACGGGTCTAT	GTACTGGAAT	ACCTGAAAG	GATGAACCGT				
7570	7580	7590	7600	7610	7620				
GTACATCTAC	GTATTAGTCA	TCGCTATTAC	CATGGTGAATG	CGGTTTGGC	AGTACATCAA				
CATGTAGATG	CATAATCAGT	AGCGATTAATG	GTACCACCTAC	GCCAAAACCG	TCATGTAGTT				
7630	7640	7650	7660	7670	7680				
TGGCGGTGA	TAGCGTTTG	ACTCACGGG	ATTTCCAAGT	CTCCACCCCA	TTGACGTCAA				
ACCCGACCT	ATCGCCAAAC	TGAGTGCCTC	TAAAGTTTCA	GAGTGGGGT	AACTGCAGTT				
7690	7700	7710	7720	7730	7740				
TGGGAGTTTG	TTTTTGGCACC	AAATCAACG	GGACTTTCCA	AAATGTCCTA	ACAACTCCGC				
ACCCTCANAC	AAAACCGTGG	TTTTTAGTTGC	CCTGAAAGGT	TTTACAGCAT	TGTTGAGGCG				
7750	7760	7770	7780	7790	7800				
CCCATTTGACG	CAAAATGGCG	GTAGCGGTGT	ACGGTGGGAG	GTCTATATAA	GCAGAGCTCT				
GGGTNACTGC	GTTTACCCCG	CATCCGCACA	TGCCACCCCTC	CAGATATATT	CGTCTCGAGA				



FIGURE 19N

pD17-hG1b

7810	7820	7830	7840	7850	7860
CTGGCTAACT	AGAGAACCCA	CTGCTTACTG	GCTTATCGAA	ATTAATACGA	CTCACTATAG
GACCGATTGA	TCTCTTGGGT	GACGAATGAC	CGAATAGCTT	TAATTATGCT	GAGTGATATC
7870	7880				
GGAGACCCAA	GCTT				
CCTCTGGGTT	CGAA				

47156

FIGURE 20

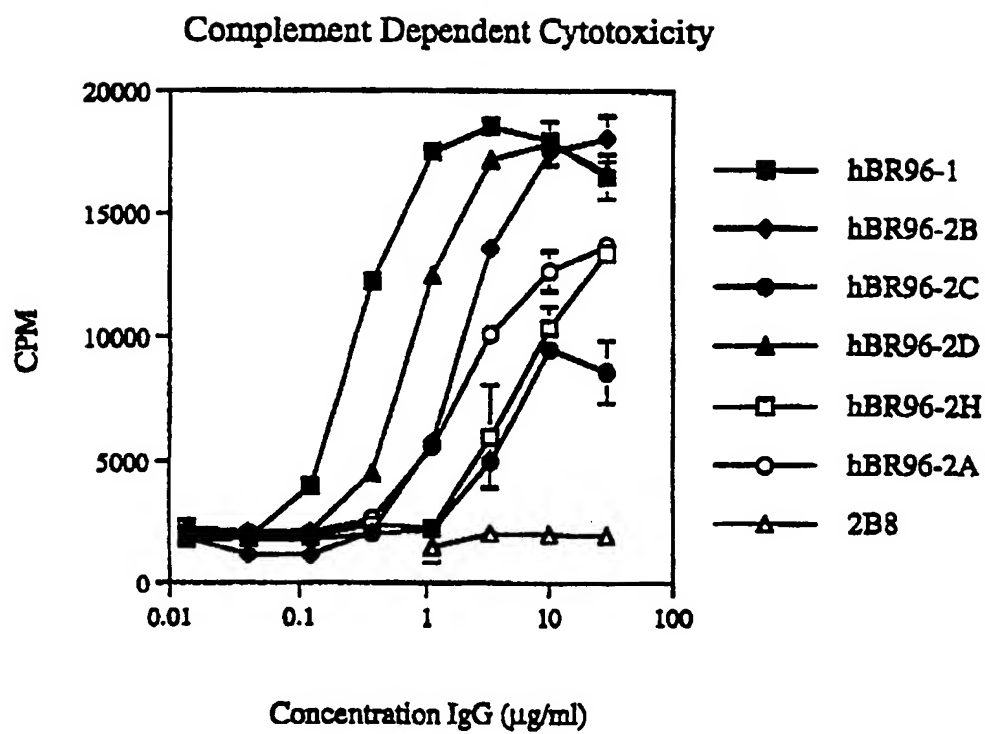


FIGURE 21

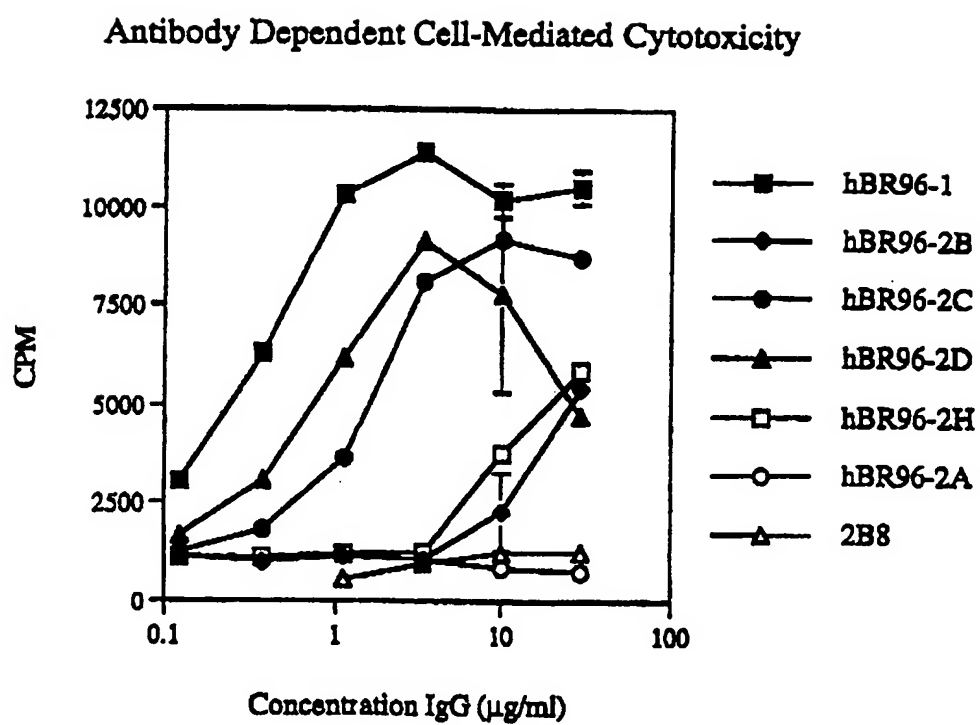
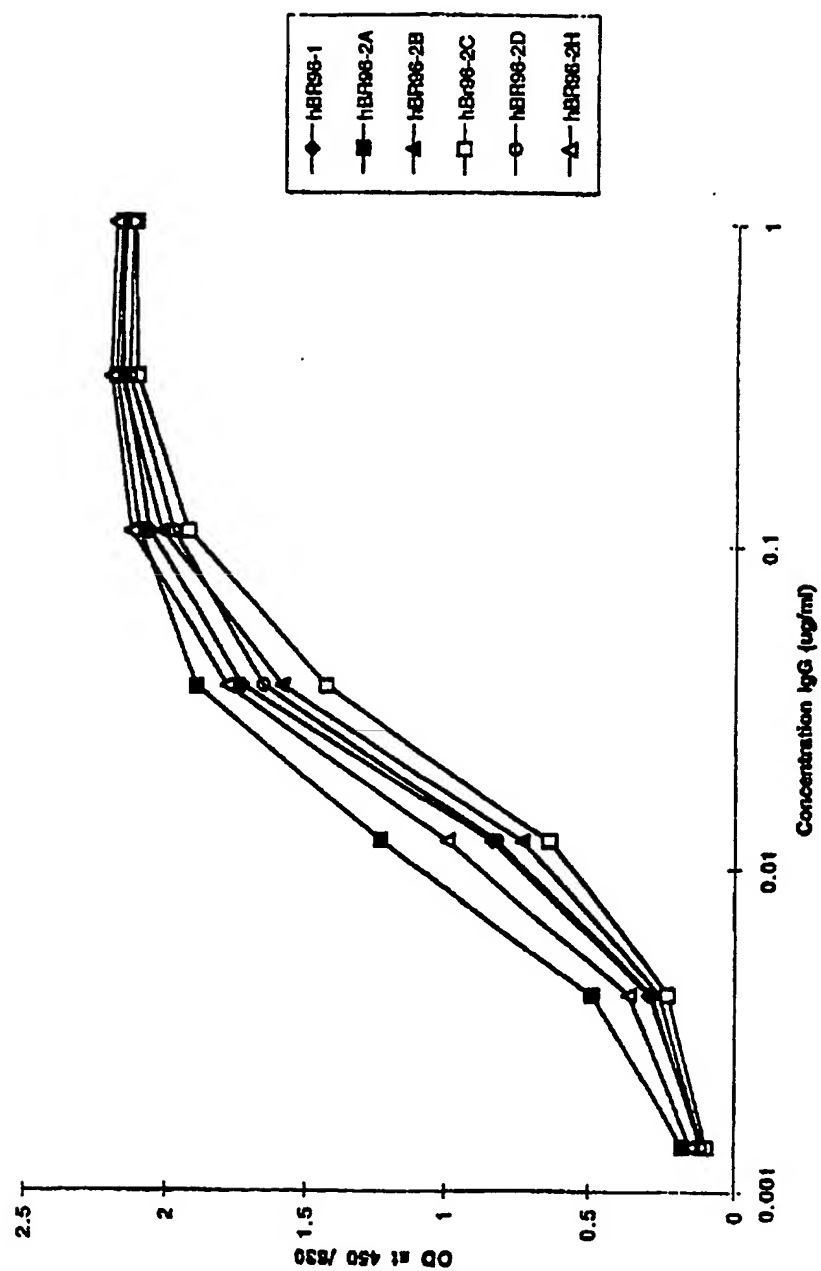


FIGURE 22

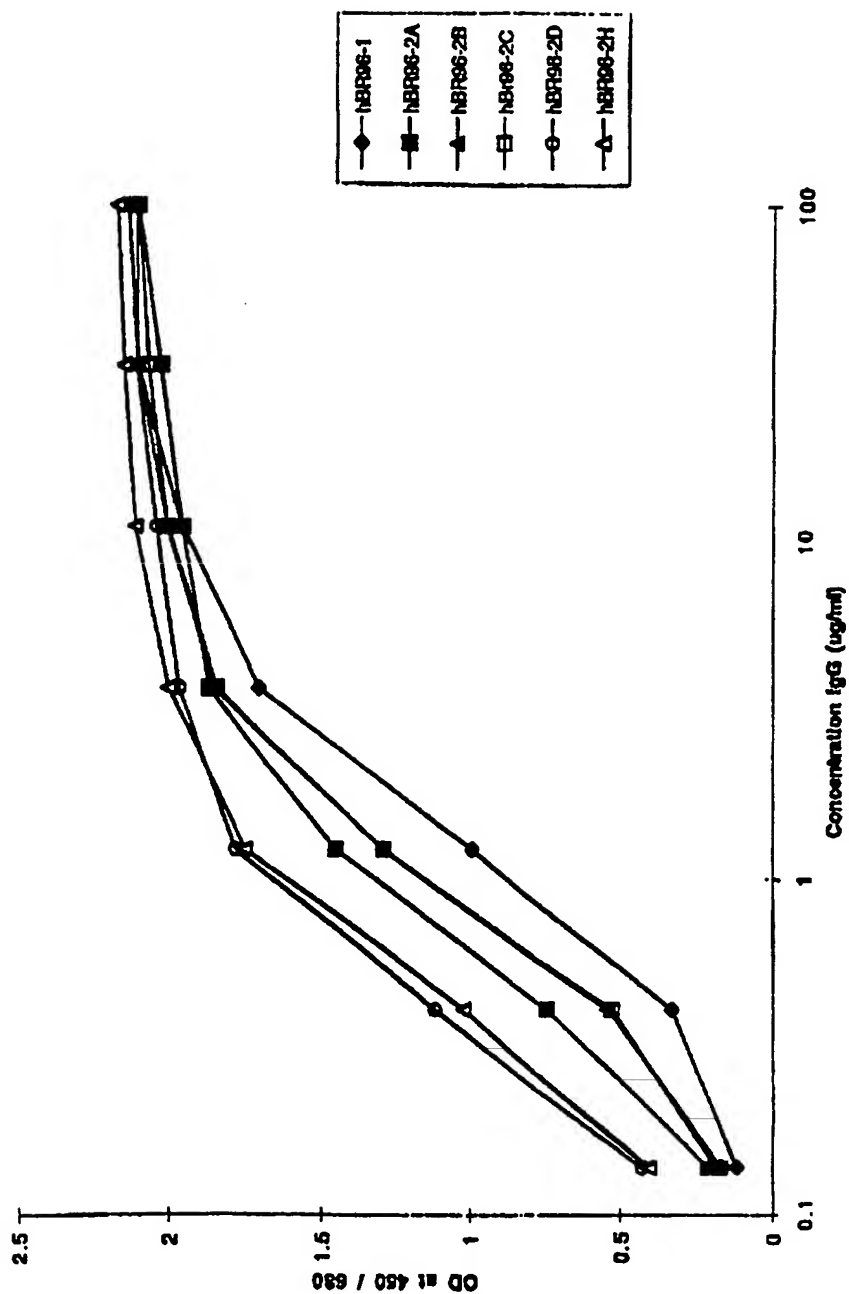
Binding activity of hBR96-2 constant region mutants on LeY-HSA



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FIGURE 23

Binding activity of hBR98-2 constant region mutants on LNFP111-BSA



51156

Figure 24

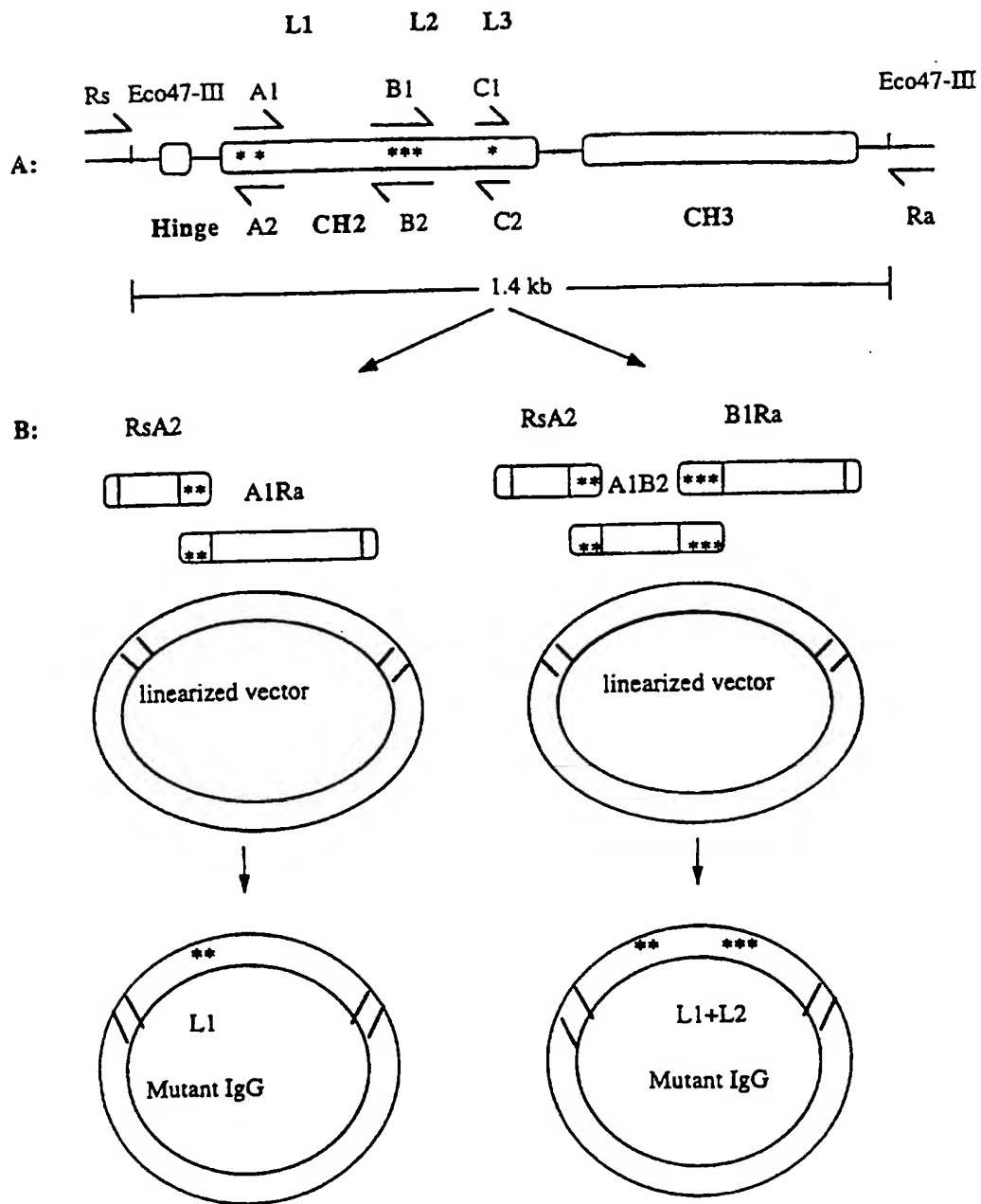


Figure 25

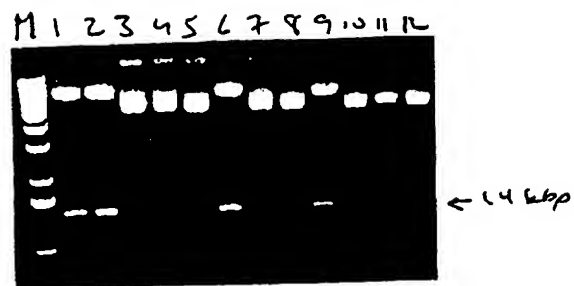


Figure 26

## hBR96-2 Heavy Chain Variable Region (VH)

1                    11                    21                    31                    41  
 EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYMYWVRQA PGKGLEWVSY  
 51                    61                    71                    81                    91  
 ISQDGDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL  
 101                    111  
 ADGAWFAYWG QGTLTVSS

## human IgG1 constant

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 STKGPSVPPL APSSKSTSGG TAALGCLVKD  
 YFPEFVTVSW NSGALTSGVH TFPVQLQSSG LYSLSSTVTV PSSSLCTQTY  
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## Figure 27

## hBR96-2A: Heavy Chain Variable Region (VH)

<sup>1</sup>EVQLVESGGG <sup>11</sup>LVQPGGSLRL <sup>21</sup>SCAASGFPFS <sup>31</sup>DYYMYWVRQA <sup>41</sup>PGKGLEWVSY  
<sup>51</sup>ISQDGDITDY <sup>61</sup>ADSVKGRFTI <sup>71</sup>SRDNAKNSLY <sup>81</sup>LQMNSLRDED <sup>91</sup>TAVYYCARGL  
<sup>101</sup>ADGAWFAYWG <sup>111</sup>QGLTVTVSS

hBR96-2A: Human Heavy Chain IgG1 Constant Region  $\Delta$ CH2

A STKGPSVPPL APSSKSTSCG TAALGCLVKD YFPEPVTVSW NSGALTSGVH  
 TTPAVLQSSG LYSLS5VTV PSSSLQTQTY ICNVNKKPSN TKVDKKVEPK  
 SCDKTHCTPP CP QPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA  
 VEWESNGQPE NNYKTTTPVL DSDGSFFLYS KLTVDKSRWQ QGNVPSCSVH  
 HEALHNHYTC KSLSLSPGK

## Figure 28

This sequence is the chi BR96 IgG1 with CH2 deleted.

1 <sup>VA</sup> EVNLVESGGG LVQPGGSLKV SCVTSGFTFS DYMYWVRQT PEKRLWVAY  
51 ISQGGDITDY PDTVGRFTI SRD<sup>CH1</sup>NAKNTLY LQMSRLKSED TAMY<sup>CH1</sup>CARGL  
101 DDGAWFAYWG QGTLVTVSVA STKGPSVFPL APSSKSTSGG TAALGCLVKD  
151 YFPEFVTVSW NSGALTSGVH TFP<sup>CH1</sup>AVLQSSG LYSLS<sup>CH1</sup>SVTV PSSSLGTQTY  
201 ICNVN<sup>CH1</sup>HKPSN TKVDK<sup>CH1</sup>RVEPK SCDK<sup>CH1</sup>HTCPP CH<sup>CH1</sup>QPREPQV YTLFFSRDEL  
251 TKNQVSLTCL VRGFYPSDIA VEWESNGQFE NNYK<sup>CH1</sup>TTPFVL DSDGSFFLYS  
301 KLTVDKSRWQ QGNVFSCSVN HEALHNHYTQ KSLSLSPGK

# INTERNATIONAL SEARCH REPORT

Intern: al Application No  
PCT/US 97/13562

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 C12N15/62 A61K39/395 A61K38/17 A61K47/48 A61K51/10  
C07K16/30 C07K16/46 C07K16/00 C12N15/13 C12N1/21  
C12N5/10 //C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. GILLIES ET AL.: "Antigen binding and biological activities of engineered mutant chimeric antibodies with human tumor specificities." HUMAN ANTIBODIES AND HYBRIDOMAS, vol. 1, no. 1, 1990, STONEHAM, MA, USA, pages 47-54, XP002050448 see the whole document --- -/--	1-8, 23-25

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

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Date of the actual completion of the international search

17 December 1997

Date of mailing of the international search report

21. 01. 98

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Nooij, F

## INTERNATIONAL SEARCH REPORT

Intern. Application No  
PCT/US 97/13562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. SCHREIBER ET AL.: "An unmodified anticarcinoma antibody, BR96, localizes to and inhibits the outgrowth of human tumors in nude mice." CANCER RESEARCH, vol. 52, no. 12, 15 June 1992, BALTIMORE, MD, USA, pages 3262-3266, XP002050449 see abstract	33,35,36
A	---	1,2,5,7, 8,11-18, 23
A	A. DUNCAN ET AL.: "The binding site for Clq on IgG." NATURE, vol. 332, no. 6166, 21 April 1988, LONDON, GB, pages 738-740, XP002050450 cited in the application see the whole document	1,2,5,7, 8
A	---	1,2,5,7, 8
A	J. LUND ET AL.: "Human Fcγ <sub>1</sub> and Fcγ <sub>2</sub> interact with distinct but overlapping sites on human IgG." THE JOURNAL OF IMMUNOLOGY, vol. 147, no. 8, 15 October 1991, BALTIMORE, MD, USA, pages 2657-2662, XP002050451 cited in the application see abstract	1,2,5,7, 8
A	---	1-8
A	Y. XU ET AL.: "Residue at position 331 in the IgG1 and IgG4 CH2 domains contributes to their differential ability to bind and activate complement." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 5, 4 February 1994, BALTIMORE, MD, USA, pages 3469-3474, XP002050452 cited in the application see abstract see discussion	1-8
A	---	1,2,5,7, 8
A	T. MICHAELSEN ET AL.: "One disulfide bond in front of the second heavy chain constant region is necessary and sufficient for effector functions of human IgG3 without a genetic hinge." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 91, no. 20, 27 September 1994, WASHINGTON, DC, USA, pages 9243-9247, XP002050453 see the whole document	1,2,5,7, 8
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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 97/13562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	L. TAN ET AL.: "Influence of the hinge region on complement activation, C1q binding, and segmental flexibility in chimeric human immunoglobulins." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 87, no. 1, January 1990, WASHINGTON, DC, USA, pages 162-166, XP002050454 see the whole document ---	1-8
A	EP 0 699 756 A (BRISTOL-MYERS SQUIBB COMPANY) 6 March 1996 cited in the application  see examples see claims -----	11-18, 23,25, 28,29, 31-52

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 97/13562

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/13562

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 26,27

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 26 represents a method of detection/diagnosis and refers forward to claim 30, which represents a method of treatment. Claim 27 refers to a method in claim 24; however, in claim 24 a product is claimed, not a method.

Remark : Although claims 1-22, 25, 28-32 and 34-36 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/13562

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 699756 A	06-03-96	AU 2834995 A	15-02-96
		CA 2155397 A	05-02-96
		JP 8191692 A	30-07-96
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